Eculizumab: 5G1.1, h5G1.1, Long-Acting Anti-C5 Monoclonal Antibody 5G1-1, Long-Acting Anti-C5 Monoclonal Antibody 5G1.1

Author: Adis International Limited,

Publication info: Drugs in Research and Development 8.1 (2007): 61-68.

ProQuest document link

Abstract (English): Eculizumab [long-acting anti-C5 monoclonal antibody 5G1- 1, 5G1.1, h5G1.1, monoclonal antibody 5G1.1, Soliris] is a monoclonal antibody that binds to the C5 complement molecule, thereby blocking the progression of the complement cascade at this point. By binding to C5, eculizumab prevents generation of the potent anaphylatoxin C5a and the cytolytic C5b-9 complex, or membrane attack complex. The compound has been filed for approval in the US and the EU for paroxysmal nocturnal haemoglobinuria (PNH), a chronic blood disease caused by a defective enzyme that leads to a lack of protective natural complement inhibitors. It is also being investigated for the treatment of asthma and transplantation. Eculizumab is a long- acting, humanised version of the anti-C5 antibody [h5G1.1]. The short-acting version is called pexelizumab. In January 2003 Alexion Pharmaceuticals executed a large-scale product supply agreement with Lonza Biologics for the long-term commercial manufacture of eculizumab. Specific terms of the agreement were not disclosed. Alexion signed an agreement with GTC Biotherapeutics in 2000 for the production of eculizumab in transgenic goats. However, this agreement no longer appears to be active. Alexion has also filed for approval for eculizumab in PNH in the EU under the centralised procedure. The EMEA has granted Alexions request for evaluation of the proposed MAA for eculizumab in PNH under the accelerated assessment procedure. This shortens the review part of the MAA procedure. Results from the phase III TRIUMPH trial have been reported; all pre- specified primary and secondary endpoints were achieved with statistical significance. The last patient completed the last visit in the SHEPARD trial in October 2006, and 12-month data is being submitted to regulatory applications. Positive preliminary interim results of 6 months of therapy have been reported. The study has been conducted to collect additional data on eculizumabs safety among haemolytic PNH patients who have undergone transfusion. The trial enrolled 97 patients in the US, Canada, Europe and Australia and includes 12 months of treatment. A pilot, open-label phase Ib trial evaluating eculizumab in 11 patients with PNH has been completed in the UK. All 11 patients in the trial chose to participate in two consecutive 1-year extension studies. One of these is still ongoing, with patients having completed at least 26 months of chronic eculizumab therapy. One- and two-year cumulative results have been presented. The positive findings further supported progression of the clinical development of eculizumab for PNH. Eculizumab has been granted orphan drug status in the US and EU for the treatment of PNH. . Phase I and II trials in rheumatoid arthritis had previously been completed. The rationale behind evaluating eculizumab in this indication was that activated terminal complement proteins appear to have a central role in the pathogenesis of immune-mediated joint inflammation, indicating that anti-C5 monoclonal antibodies may have therapeutic potential for the treatment of rheumatoid arthritis. A phase I pilot trial was completed in patients with dermatomyositis, an inflammatory skin and muscle disorder, for which eculizumab has orphan drug status in the US. The US FDA granted eculizumab fast-track designation in membranous glomerulonephritis in early 2000 and the drug has orphan drug status in the US for idiopathic membranous glomerular nephropathy. Both long-acting and short-acting versions of anti- C5 monoclonal antibody 5G1.1 are being developed by Alexion Pharmaceuticals in the US, with the use of technology licensed from Stanford University. Eculizumab is covered by US Patent No. 6 355 245, which extends to C5-specific monoclonal antibodies for the treatment of inflammatory diseases. The Japanese Patent Office issued patent number 3 734 266 for eculizumab in Japan in February 2006. The patent includes the use of eculizumab to inhibit complement activation in humans, the key mechanism of action used for the treatment of PNH. Corresponding patents have been issued or are pending in Europe, Canada, Australia and other key markets.

Subject: Statistics;Joint diseases;Membrane attack complex;Transfusion;Clinical trials;Dermatomyositis;Inflammatory diseases;Nephropathy;Drugs;Anaphylatoxin C5a;Skin;Membranous glomerulonephritis;Monoclonal antibodies;Patents;Muscles;Asthma;Enzymes;complement component C5;Drug development;Inflammation;Blood;Rheumatoid arthritis;Complement activation;Pharmaceuticals;Complement inhibitors

Language: English

Document type: Journal Article

Publication title: Drugs in Research and Development

Source details: Drugs in Research and Development [Drugs Res. Develop.]. Vol. 8, no. 1, pp. 61-68. 2007.

Volume: 8

Issue: 1

Pagination: 61-68

Page count: 8

ISSN: 1174-5886

Publisher: Adis International Ltd., 41 Centorian Drive Private Bay 65901, Mairangi Bay Auckland 10 New Zealand, [mailto:sportsmed@adis.co.nz], [URL:http://www.adis.com]

Subfile: Immunology Abstracts

Publication date: 2007

Date revised: 2007-07-01

Accession number: 7479799

Document URL: http://dialog.proquest.com/professional/docview/19694204?accountid=157282

First available: 2010-04-29

Updates: 2015-03-262015-04-01

Database: ProQuest Biological &Health Science Professional (1971 - current)

Contact ProQuest
Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

Alexion completes enrollment in Ph II kidney disease trial

Publication info: Marketletter (Feb 25, 2002): NA.
ProQuest document link

Abstract (English): Alexion has completed enrollment in a Phase II study of its complement C5 inhibitor eculizumab (formerly 5G1.1) in patients with the kidney disease membranous nephritis. The drug has just been granted orphan status in the USA for this indication.

Full text: Alexion has completed enrollment in a Phase II study of its complement C5 inhibitor eculizumab (formerly 5G1.1) in patients with the kidney disease membranous nephritis. The drug has just been granted orphan status in the USA for this indication.

Subject: Drugs

Location: United States

Company / organization: Alexion Pharmaceuticals Inc.

Identifier (keyword): Trade, ALXN

Event classification: 331: Product development

NAICS classification: 325412: Pharmaceutical Preparation Manufacturing

Product classification: 2834000: Pharmaceutical Preparations

SIC classification: 2834: Pharmaceutical preparations

Publication title: Marketletter

Pagination: NA

Publication date: Feb 25, 2002

Publisher: Marketletter Publications Ltd.

Journal subject: Business, international

ISSN: 0951-3175

Journal code: 0VAC

Source type: Newsletter

Language of publication: English

Document type: Brief Article

Source attribution: Gale PROMT, © Publisher specific

Accession number: 83149784

Document URL: http://dialog.proquest.com/professional/docview/678220943?accountid=157282

Copyright: Copyright 2002 Gale, Cengage Learning. All rights reserved.COPYRIGHT 2002 Marketletter Publications Ltd.

Date created: 2010-04-28

First available: 2010-07-25

Database: Gale Group PROMT® (1972 - current)

Contact ProQuest
Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

Alexion Completes Enrollment in Phase II Membranous Nephritis Clinical Trial with 5G1.1; - 5G1.1 Receives Orphan Drug Designation in Membranous Nephritis -

Publication info: PR Newswire 20 Feb 2002: NA.

ProQuest document link

Abstract (English): CHESHIRE, Conn. -- Alexion Pharmaceuticals Inc. announced today that it has completed enrollment in its first Phase II trial in patients suffering from a severe form of kidney disease known as membranous nephritis, with its anti-inflammatory C5 Inhibitor antibody, 5G1.1, now called eculizumab. Separately, Alexion announced that it has received orphan drug designation from the Food and Drug Administration for the use of eculizumab, if approved, in patients with membranous nephritis.
This Phase II multi-center, double-blinded, randomized, placebo-controlled study examines the safety and clinical efficacy of repeated dosing of eculizumab during a four month treatment period. Approximately 115 membranous nephritis patients were enrolled in the United States.

Full text: CHESHIRE, Conn. -- Alexion Pharmaceuticals Inc. announced today that it has completed enrollment in its first Phase II trial in patients suffering from a severe form of kidney disease known as membranous nephritis, with its anti-inflammatory C5 Inhibitor antibody, 5G1.1, now called eculizumab. Separately, Alexion announced that it has received orphan drug designation from the Food and Drug Administration for the use of eculizumab, if approved, in patients with membranous nephritis.

This Phase II multi-center, double-blinded, randomized, placebo-controlled study examines the safety and clinical efficacy of repeated dosing of eculizumab during a four month treatment period. Approximately 115 membranous nephritis patients were enrolled in the United States. Alexion also announced that, as a part of its comprehensive development plan for eculizumab, it has already commenced dosing in a follow-on open label extension study of eculizumab in these patients, designed to examine long-term safety and tolerability.

"Completing enrollment in the first eculizumab Phase II trial in patients suffering from membranous nephritis brings us closer to our objective of expanding the profile of eculizumab into a variety of chronic autoimmune opportunities," stated Leonard Bell, M.D., President and Chief Executive Officer of Alexion. "Once the four month treatment period and all follow-up patient visits are completed, we expect to complete data collection and then lock the database for subsequent analysis of this Phase II trial. Additionally, we are encouraged by the orphan drug designation as it may provide an innovative therapy for this small, but underserved patient population, while potentially providing us with development and commercial advantages."

According to the National Kidney Foundation, more than 260,000 Americans suffer from chronic kidney failure, and more than 50,000 people die each year because of kidney disease. Membranous nephritis is the most common cause of the nephrotic syndrome in adults worldwide, and is characterized by the abnormal excretion of large amounts of protein in the urine (proteinuria), which can cause a number of severe medical complications. Membranous nephritis is a form of kidney disease that is believed to be caused by a chronic autoimmune disorder in which antibodies and complement target the kidney. It is estimated that there are approximately 100,000 - 200,000 people currently afflicted with idiopathic membranous nephritis in the United States. Currently, in such a severe disease population, more than 30% of the patients are expected to progress to renal failure, which may require dialysis or transplantation.

Alexion is engaged in the discovery and development of therapeutic products aimed at treating patients with a wide array of severe disease states, including cardiovascular and autoimmune disorders, inflammation and cancer. Alexion's two lead product candidates, pexelizumab and 5G1.1, are currently in eight clinical development programs. Alexion is developing pexelizumab, an antibody fragment, in collaboration with Procter &Gamble Pharmaceuticals. Together the firms have initiated a Phase III clinical study with pexelizumab in cardiopulmonary bypass patients, and are currently conducting two large Phase II studies with pexelizumab in

acute myocardial infarction patients. Alexion's other lead product candidate, 5G1.1, has completed a Phase II trial for the treatment of rheumatoid arthritis. 5G1.1 is also in Phase II trials for the treatment of membranous nephritis and for lupus nephritis, and in earlier stage clinical trials for the treatment of dermatomyositis and pemphigoid. Additionally, through its wholly owned subsidiary, Alexion Antibody Technologies, Inc., Alexion is engaged in discovering and developing a portfolio of additional antibody therapeutics targeting severe unmet medical needs. This press release and further information about Alexion Pharmaceuticals, Inc. can be found on the World Wide Web at: http://www.alexionpharm.com/.

This news release contains forward-looking statements. Such statements are subject to certain factors which may cause Alexion's plans to differ or results to vary from those expected, including the results of pre-clinical or clinical studies (including termination or delay in clinical programs or inability to move forward to the next Phase of clinical development), the need for additional research and testing, delays in developing or arranging satisfactory manufacturing capability, inability to access capital and funding on a timely basis and on favorable terms, delays in development of or adverse changes in status of commercial relationships, the possibility that favorable results of earlier clinical trials are not predictive of safety and efficacy results in larger clinical trials, dependence on Procter &Gamble Pharmaceuticals for performance of development and commercial matters related to pexelizumab, the risk that third parties won't agree to license us on reasonable terms their intellectual property necessary for us to develop and commercialize our products, and a variety of other risks set forth from time to time in Alexion's filings with the Securities and Exchange Commission, including but not limited to the risks discussed in Alexion's Annual Report on Form 10-K for the year ended July 31, 2001. Except in special circumstances in which a duty to update arises under law when prior disclosure becomes materially misleading in light of subsequent events, Alexion does not intend to update any of these forward-looking statements to reflect events or circumstances after the date hereof or to reflect the occurrence of unanticipated events.

Contacts:

Alexion Pharmaceuticals, Inc.
Leonard Bell, M.D.
President &CEO
(203) 272-2596
Noonan/Russo Communications, Inc.
Ernie Knewitz (Media)
(212) 696-4455 Ext. 204
Rx Communications
Rhonda Chiger (Investor)
917-322-2569

MAKE YOUR OPINION COUNT - Click Here
http://tbutton.prnewswire.com/prn/11690X36378205

Contact: Leonard Bell, M.D., President &CEO of Alexion Pharmaceuticals, Inc., +1-203-272-2596; media - Ernie Knewitz of Noonan-Russo Communications, Inc., +1-212-696-4455 Ext. 204; or investor - Rhonda Chiger of Rx Communications, +1-917-322-2569, both for Alexion Pharmaceuticals Inc.

Subject: Drugs;Pharmaceutical industry

Company / organization: Alexion Pharmaceuticals Inc.

Identifier (keyword): Trade, ALXN

NAICS classification: 325412: Pharmaceutical Preparation Manufacturing

Product classification: 2834000: Pharmaceutical Preparations

SIC classification: 2834: Pharmaceutical preparations

Publication title: PR Newswire

Pagination: NA

Publication date: Feb 20, 2002

Publisher: PR Newswire Association LLC

Journal subject: Business, News, opinion and commentary

Journal code: 4PRN

Source type: Newswire

Language of publication: English

Document type: Magazine/Journal

Source attribution: Gale Group Trade &Industry, © Publisher specific

Accession number: 83071115

Document URL: http://dialog.proquest.com/professional/docview/1054819938?accountid=157282

Copyright: Copyright 2002 Gale, Cengage Learning. All rights reserved.COPYRIGHT 2002 PR Newswire Association LLC

Date created: 2012-08-07

Updates: 2012-09-22

First available: 2012-09-22

Database: Gale Group Trade &Industry Database™ (1976 - current)

Contact ProQuest

Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

Alexion issued C5 complement inhibitor patent for inflammatory diseases

Publication info: ESPICOM Pharmaceutical and Medical Device News (Mar 19, 2002).
ProQuest document link

Full text: Alexion Pharmaceuticals has been issued US Patent No. 6,355,245 entitled: "C5-Specific Antibodies for the Treatment of Inflammatory Diseases," from the US PTO. The issued claims relate to the company's C5 complement inhibitor programme and cover the composition and use of Alexion's lead drug candidates, eculizumab (formerly known as 5G1.1) and pexelizumab, as well as other antibodies that bind to C5 and effectively inhibit its inflammatory activity.

Subject: Pharmaceutical

Language: English

Document type: Article

Publication title: ESPICOM Pharmaceutical and Medical Device News

Publication type: Newsletter

Publication date: Mar 19, 2002

Source attribution: ESPICOM Pharmaceutical and Medical Device News, © Publisher specific

Accession number: 00068816

Document URL: http://dialog.proquest.com/professional/docview/764831846?accountid=157282

First available: 2010-11-15

Database: ESPICOM Pharmaceutical &Medical Device News (2000 - current)

Contact ProQuest
Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

Alexion Issued Key C5 Complement Inhibitor Patent for Inflammatory Diseases

Publication info: PR Newswire 15 Mar 2002: NYF00215032002.
ProQuest document link

Abstract (English): CHESHIRE, Conn. -- Alexion Pharmaceuticals, Inc. today announced that it has been issued United States Patent No. 6,355,245 entitled "C5-Specific Antibodies for the Treatment of Inflammatory Diseases," from the United States Patent and Trademark Office. The issued claims relate to the company's C5 Complement Inhibitor program and cover the composition and use of Alexion's lead drug candidates, eculizumab (formerly known as 5G1.1) and pexelizumab, as well as other antibodies that bind to C5 and effectively inhibit its inflammatory activity.
"Issuance of the patent further supports our aggressive pursuit to develop pexelizumab and eculizumab in a variety of severe acute and chronic inflammatory conditions and is an important element in our commercialization strategy," stated Leonard Bell, M.D., President and Chief Executive Officer of Alexion.

Full text: CHESHIRE, Conn. -- Alexion Pharmaceuticals, Inc. today announced that it has been issued United States Patent No. 6,355,245 entitled "C5-Specific Antibodies for the Treatment of Inflammatory Diseases," from the United States Patent and Trademark Office. The issued claims relate to the company's C5 Complement Inhibitor program and cover the composition and use of Alexion's lead drug candidates, eculizumab (formerly known as 5G1.1) and pexelizumab, as well as other antibodies that bind to C5 and effectively inhibit its inflammatory activity.
"Issuance of the patent further supports our aggressive pursuit to develop pexelizumab and eculizumab in a variety of severe acute and chronic inflammatory conditions and is an important element in our commercialization strategy," stated Leonard Bell, M.D., President and Chief Executive Officer of Alexion. "This patent represents the cornerstone of our proprietary position in C5 complement inhibition. It is also significant as it comes at a time when we have achieved several key milestones in our clinical programs including commencement of enrollment in the Phase III pivotal PRIMO-CABG trial and completion of enrollment in the Phase II COMPLY myocardial infarction trial with pexelizumab, and in parallel, the initiation of our first Phase IIb trial in rheumatoid arthritis and the completion of enrollment of the Phase II membranous nephritis trial with eculizumab."
Alexion is engaged in the discovery and development of therapeutic products aimed at treating patients with a wide array of severe disease states, including cardiovascular and autoimmune disorders, inflammation and cancer. Alexion's two lead product candidates, pexelizumab and eculizumab, are currently in eight clinical development programs. Alexion is developing pexelizumab, an antibody fragment, in collaboration with Procter &Gamble Pharmaceuticals. Together the firms have initiated a Phase III clinical study with pexelizumab in cardiopulmonary bypass patients, and are currently conducting two large Phase II studies with pexelizumab in acute myocardial infarction patients. Alexion's other lead product candidate, eculizumab, is being studied in a Phase IIb trial for the treatment of rheumatoid arthritis. Eculizumab is also in Phase II trials for the treatment of membranous nephritis and for lupus nephritis, and in earlier stage clinical trials for the treatment of dermatomyositis and pemphigoid. Additionally, through its wholly owned subsidiary, Alexion Antibody Technologies, Inc., Alexion is engaged in discovering and developing a portfolio of additional antibody therapeutics targeting severe unmet medical needs. This press release and further information about Alexion Pharmaceuticals, Inc. can be found on the World Wide Web at: http://www.alexionpharm.com/ .
This news release contains forward-looking statements. Such statements are subject to certain factors which may cause Alexion's plans to differ or results to vary from those expected, including the results of pre-clinical or clinical studies (including termination or delay in clinical programs or inability to move forward to the next Phase of clinical development), the need for additional research and testing, delays in developing or arranging

satisfactory manufacturing capability, inability to access capital and funding on a timely basis and on favorable terms, delays in development of or adverse changes in status of commercial relationships, the possibility that favorable results of earlier clinical trials are not predictive of safety and efficacy results in larger clinical trials, dependence on Procter &Gamble Pharmaceuticals for performance of development and commercial matters related to pexelizumab, the risk that third parties won't agree to license us on reasonable terms their intellectual property necessary for us to develop and commercialize our products, and a variety of other risks set forth from time to time in Alexion's filings with the Securities and Exchange Commission, including but not limited to the risks discussed in Alexion's Annual Report on Form 10-K for the year ended July 31, 2001. Except in special circumstances in which a duty to update arises under law when prior disclosure becomes materially misleading in light of subsequent events, Alexion does not intend to update any of these forward-looking statements to reflect events or circumstances after the date hereof or to reflect the occurrence of unanticipated events.

CONTACT:
Alexion Pharmaceuticals, Inc.
Stephen P. Squinto, Ph.D.
Executive Vice President and Head of Research
(203) 272-2596
Noonan/Russo Communications, Inc.
Ernie Knewitz (Media)
(212) 696-4455 Ext. 204
Rx Communications
Rhonda Chiger (Investor)
917-322-2569
MAKE YOUR OPINION COUNT - Click Here
http://tbutton.prnewswire.com/prn/11690X75229070

Contact: Stephen P. Squinto, Ph.D., Executive Vice President and Head of Research of Alexion Pharmaceuticals, +1-203-272-2596, or Media, Ernie Knewitz of Noonan-Russo Communications, +1-212-696-4455, ext. 204, or Investor, Rhonda Chiger of Rx Communications +1-917-322-2569
Website: http://www.noonanrusso.com/index.html

Subject: Drugs;Pharmaceutical industry

Location: United States

Company / organization: Alexion Pharmaceuticals Inc.

Identifier (keyword): Trade, ALXN

NAICS classification: 325412: Pharmaceutical Preparation Manufacturing

Product classification: 2834000: Pharmaceutical Preparations

SIC classification: 2834: Pharmaceutical preparations

Publication title: PR Newswire

Pagination: NYF00215032002

Publication date: Mar 15, 2002

Publisher: PR Newswire Association LLC

Journal subject: Business, News, opinion and commentary

Journal code: 4PRN

Source type: Newswire

Language of publication: English

Document type: Magazine/Journal

Source attribution: Gale Group New Product Announcements Plus, © Publisher specific

Accession number: 83784979

Document URL: http://dialog.proquest.com/professional/docview/1072327038?accountid=157282

Copyright: Copyright 2002 Gale, Cengage Learning. All rights reserved.COPYRIGHT 2002 PR Newswire Association LLC

Date created: 2010-11-27

Updates: 2012-09-25

First available: 2012-09-25

Database: Gale Group New Product Announcements / Plus® (1985 - current)

Contact ProQuest

Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

Alexion Pharmaceuticals Reports Second Quarter and First Half Results

Publication info: PR Newswire [New York] 08 Mar 2002: 1.
ProQuest document link

Abstract (summary): "Alexion achieved several very significant milestones in the second quarter, including the initiation of the pexelizumab pivotal Phase III PRIMO- CABG trial, completion of patient enrollment in the first of our two acute myocardial infarction Phase II trials with pexelizumab, and the successful restructuring of our collaboration agreement with P&G. We are excited that the revised collaboration with P&G allows Alexion to share in decision-making and development and commercial responsibilities for this important product and provides that we and P&G will share the gross margin on sales in the U.S., on an approximately equal basis," said David Keiser, Executive VP and Chief Operating Officer of Alexion. "Our pipeline has made tremendous progress, also in other areas during the quarter, most noteworthy, being the initiation of a Phase IIb trial of eculizumab, formerly 5G1.1, in rheumatoid arthritis patients."

Alexion is engaged in the discovery and development of therapeutic products aimed at treating patients with a wide array of severe disease states, including cardiovascular and autoimmune disorders, inflammation and cancer. Alexion's two lead product candidates, pexelizumab and eculizumab, are currently in eight clinical development programs. Alexion is developing pexelizumab, an antibody fragment, in collaboration with Procter &Gamble Pharmaceuticals. Together the firms have initiated a Phase III clinical study with pexelizumab in cardiopulmonary bypass patients, and are currently conducting two large Phase II studies with pexelizumab in acute myocardial infarction patients. Alexion's other lead product candidate, eculizumab, is being studied in a Phase IIb trial for the treatment of rheumatoid arthritis. Eculizumab is also in Phase II trials for the treatment of membranous nephritis and for lupus nephritis, and in earlier stage clinical trials for the treatment of dermatomyositis and pemphigoid. Additionally, through its wholly owned subsidiary, Alexion Antibody Technologies, Inc., Alexion is engaged in discovering and developing a portfolio of additional antibody therapeutics targeting severe unmet medical needs. This press release and further information about Alexion Pharmaceuticals, Inc. can be found on the World Wide Web at: http:// www.alexionpharm.com.

Full text: - Quarter Highlighted by Successful Revision of Pexelizumab
Collaboration With Procter &Gamble Pharmaceuticals and Commencement
Of Phase III Pivotal PRIMO-CABG Trial -

CHESHIRE, Conn., March 8 /PRNewswire-FirstCall/ -- Alexion Pharmaceuticals, Inc. (Nasdaq: ALXN) today announced financial results for its second fiscal quarter and half year ended January 31, 2002.

In its second quarter ended January 31, 2002, Alexion received revenues amounting to $3.38 million compared to $3.17 million received in the same period ended January 31, 2001. Revenues were slightly higher than in the same period last year primarily due to receipt of a $2 million milestone payment from our pexelizumab development partner, Procter &Gamble Pharmaceuticals (P&G) for the initiation of our pivotal Phase III clinical trial in CABG patients, called "PRIMO-CABG."

The Company's research and development expenses for the three month period were $15.04 million compared to $9.40 million for the same period last year. The $5.64 million increase resulted principally from greater manufacturing costs associated with our lead C5 inhibitor candidates, pexelizumab and eculizumab (formerly 5G1.1), and our incurred ongoing pexelizumab Phase III CABG clinical trial costs. As part of the revised collaboration agreement with P&G signed in December 2001, it was agreed that the Company would bear the first 50% of the projected clinical trial costs for the on-going Phase III PRIMO-CABG study and P&G would bear the second half. As such, in the quarter, we recognized a one-time $3.10 million expense for unbilled reimbursable contract costs associated with manufacturing of pexelizumab for Phase III trials which would have been paid by P&G under the original agreement.

General and administrative expenses were $1.84 million for the quarter compared to $1.97 million for the same period last year, mostly as a result of decreased recruiting costs. Accordingly, total operating expenses increased to $16.88 million for the quarter compared to $11.37 million in the three month period ended January 31, 2001, excluding the non-cash amortization of goodwill. The Company posted interest income for the quarter of $3.92 million compared to $6.54 million for the same period last year. The decrease was primarily due to reduced market interest rates. Interest expense, primarily on the Company's $120 million convertible subordinated notes, was $1.93 million for the quarter versus $1.95 million for the same period last year. A state income tax credit was recognized for the three months ended January 31, 2002 representing a state tax benefit of approximately $700,000.

As a result of the above factors, the Company incurred a net loss for the quarter of $10.81 million, or $0.60 per share versus a net loss of $3.61 million or $0.20 per common share for the same three month period in 2001. Taking into account the non-cash amortization of goodwill related to the Company's acquisition of Prolifaron in September 2000, the Company's total net loss amounted to $4.49 million or $0.25 per common share for the quarter ended January 31, 2001.

For the six months ended January 31, 2002, Alexion's revenues were $5.24 million compared to $6.57 million for the same period last year. The $1.33 million decrease in contract research revenues occurred, despite receipt of the $2 million milestone payment, primarily because clinical trial and clinical manufacturing payments from P&G were lower resulting from last year's completion of the Phase IIb pexelizumab CABG study.

Research and development expenditures were $24.71 million for the first half of this fiscal year compared to $20.32 million for the six months ended January 31, 2001. The increase was, again, attributable to higher clinical manufacturing costs associated with our lead C5 inhibitor candidates, pexelizumab and eculizumab, and incurred clinical trial costs for the Company's ongoing pexelizumab Phase III CABG trial.

General and administrative expenses increased slightly to $3.44 million for the six months from $3.35 million for the same period last year mostly as a result of higher payroll costs from added personnel. Accordingly, total operating expenses increased to $28.15 million for the half year compared to $23.67 million, without taking into account last year's acquisition related non-cash charges, in the six month period ended January 31, 2001.

The Company posted interest income for the past six months of $7.46 million compared to $9.34 million for the same period last year. The decrease was primarily due to reduced market interest rates. Interest expense was $3.85 million for the quarter versus $3.95 million for the same period last year. A state income tax credit was recognized in the second quarter ended January 31, 2002 representing a state tax benefit of approximately $700,000.

Net loss for the six months increased to $18.60 million or $1.03 per share from a net loss of $11.70 million or $0.70 per share recorded for the same six month period last year, exclusive of the impact of non-cash charges. Last year's non-cash charges related to Alexion's acquisition of Prolifaron in September 2000 in which the Company recognized a $21.0 million one-time first quarter charge allocated to in-process research and development projects and $1.23 million charge for amortization of goodwill. In addition, the Company recorded a $9.12 million non-cash charge for the cumulative effect of adoption of SAB 101. Taking into account these non-cash charges, the Company's total net loss including operating expenses amounted to $43.05 million or $2.58 per common share for the six months ended January 31, 2001.

As of January 31, 2002 Alexion had approximately $341 million in cash, cash equivalents and marketable securities.

"Alexion achieved several very significant milestones in the second quarter, including the initiation of the pexelizumab pivotal Phase III PRIMO- CABG trial, completion of patient enrollment in the first of our two acute myocardial infarction Phase II trials with pexelizumab, and the successful restructuring of our collaboration agreement with P&G. We are excited that the revised collaboration with P&G allows Alexion to share in decision-making and development and commercial responsibilities for this important product and provides that

we and P&G will share the gross margin on sales in the U.S., on an approximately equal basis," said David Keiser, Executive VP and Chief Operating Officer of Alexion. "Our pipeline has made tremendous progress, also in other areas during the quarter, most noteworthy, being the initiation of a Phase IIb trial of eculizumab, formerly 5G1.1, in rheumatoid arthritis patients."

As a result of the revised collaboration with P&G, of which terms include an approximate 50% share of gross margin on U.S. sales and a royalty on sales in the rest of the world, Alexion will incur approximately 50% of all Phase III clinical trial, product development, manufacturing, and commercialization costs necessary for the potential approval and marketing of pexelizumab in the U.S. Additionally, as part of the agreement, Alexion will generally forgo further research and development support payments from P&G. As a result, Alexion anticipates its contract research revenues will not exceed $7.0 million for the current fiscal year, and operating expenses will increase significantly going forward, reaching $78 to $82 million by the end of our fiscal year in July 31, 2002. Net loss for the year ending July 31, 2002 is expected to be in the $65 to $70 million range.

Alexion is engaged in the discovery and development of therapeutic products aimed at treating patients with a wide array of severe disease states, including cardiovascular and autoimmune disorders, inflammation and cancer. Alexion's two lead product candidates, pexelizumab and eculizumab, are currently in eight clinical development programs. Alexion is developing pexelizumab, an antibody fragment, in collaboration with Procter &Gamble Pharmaceuticals. Together the firms have initiated a Phase III clinical study with pexelizumab in cardiopulmonary bypass patients, and are currently conducting two large Phase II studies with pexelizumab in acute myocardial infarction patients. Alexion's other lead product candidate, eculizumab, is being studied in a Phase IIb trial for the treatment of rheumatoid arthritis. Eculizumab is also in Phase II trials for the treatment of membranous nephritis and for lupus nephritis, and in earlier stage clinical trials for the treatment of dermatomyositis and pemphigoid. Additionally, through its wholly owned subsidiary, Alexion Antibody Technologies, Inc., Alexion is engaged in discovering and developing a portfolio of additional antibody therapeutics targeting severe unmet medical needs. This press release and further information about Alexion Pharmaceuticals, Inc. can be found on the World Wide Web at: http:// www.alexionpharm.com.

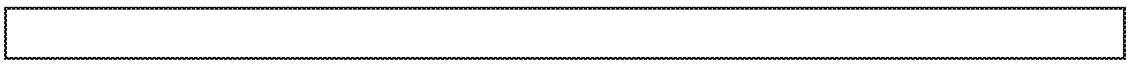
This news release contains forward-looking statements. Such statements are subject to certain factors which may cause Alexion's plans to differ or results to vary from those expected, including the results of pre-clinical or clinical studies (including termination or delay in clinical programs or inability to move forward to the next Phase of clinical development), the need for additional research and testing, delays in developing or arranging satisfactory manufacturing capability, inability to access capital and funding on a timely basis and on favorable terms, delays in development of or adverse changes in status of commercial relationships, the possibility that favorable results of earlier clinical trials are not predictive of safety and efficacy results in larger clinical trials, dependence on Procter &Gamble Pharmaceuticals for performance of development and commercial matters related to pexelizumab, the risk that third parties won't agree to license us on reasonable terms their intellectual property necessary for us to develop and commercialize our products, and a variety of other risks set forth from time to time in Alexion's filings with the Securities and Exchange Commission, including but not limited to the risks discussed in Alexion's Annual Report on Form 10-K for the year ended July 31, 2001. Except in special circumstances in which a duty to update arises under law when prior disclosure becomes materially misleading in light of subsequent events, Alexion does not intend to update any of these forward- looking statements to reflect events or circumstances after the date hereof or to reflect the occurrence of unanticipated events.

ALEXION PHARMACEUTICALS, INC.

Selected Financial Data

Statements of Operations

(dollars in thousands except per share amounts)



Quarter ended Quarter ended
January 31, January 31,
2002 2001 2001
Pro forma -a)
REVENUES $3,380 $3,174

$3,174

OPERATING EXPENSES:

Research and Development 15,043 9,399
9,399
General and Administrative 1,836 1,971

1,971

In-Process Research

and Development (IPRD) -- --
--
Amortization of Goodwill -- --
878
Total Operating Expenses 16,879 11,370
12,248
OPERATING INCOME (LOSS) (13,499) (8,196)
(9,074)
Interest Income 3,918 6,536
6,536
Interest Expense (1,929) (1,948)
(1,948)
State Tax Benefit 700 --

--
NET INCOME (LOSS) $(10,810) $(3,608) -a)

$(4,486)
BASIC AND DILUTED NET INCOME

(LOSS) PER COMMON SHARE $(0.60) $(0.20) -a)

$(0.25)
SHARES USED IN COMPUTING BASIC
AND DILUTED NET INCOME (LOSS)

PER COMMON SHARE 18,119 17,999
17,999
-a) excludes non-cash IPRD and Amortization of Goodwill
ALEXION PHARMACEUTICALS, INC.

Selected Financial Data
Statements of Operations
(dollars in thousands except per share amounts)
Six Months ended Six Months ended
January 31, January 31,

2002 2001 2001
Pro forma -a)
REVENUES $5,240 $6,573

$6,573
OPERATING EXPENSES:

Research and Development 24,714 20,322
20,322
General and Administrative 3,435 3,349

3,349
In-Process Research

and Development (IPRD) -- --

21,000
Amortization of Goodwill -- --
1,227
Total Operating Expenses 28,149 23,671
45,898
OPERATING INCOME (LOSS) (22,909) (17,098)
(39,325)
Interest Income 7,456 9,344
9,344
Interest Expense (3,846) (3,946)
(3,946)
State Tax Benefit 700 --

--

Cumulative Effect of

Adoption of SAB 101 -- --
(9,118)
NET INCOME (LOSS) $(18,599) $(11,700) -a)

$(43,045)

BASIC AND DILUTED NET INCOME

(LOSS) PER COMMON SHARE $(1.03) $(0.70) -a)

$(2.58)

SHARES USED IN COMPUTING BASIC AND

DILUTED NET INCOME (LOSS) PER

COMMON SHARE 18,114 16,661

16,661

- a) excludes non-cash IPRD, Amortization of Goodwill, and Cumulative

Effect of Adoption of SAB 101

Balance Sheet Data

(dollars in thousands)
January 31, January 31, July 31,

2002 2001 2001
(unaudited) (unaudited) (audited)
Cash, cash equivalents and
marketable securities $340,939 $369,904
$355,274
Total Assets $383,515 $414,515
$400,259
Net Stockholders' Equity $242,368 $282,214

$260,408
Contacts: Alexion Pharmaceuticals, Inc.
David Keiser
Executive Vice President &COO
(203) 272-2596
Noonan/Russo Communications, Inc. Rx Communications

Ernie Knewitz (Media) Rhonda Chiger (Investor)
(212) 696-4455 Ext. 204 917-322-2569
MAKE YOUR OPINION COUNT - Click Here

http://tbutton.prnewswire.com/prn/11690X78322878 SOURCE Alexion Pharmaceuticals, Inc.

References

Message No: Industry: MEDICAL/PHARMACEUTICALS;

Company / organization: Company / organization Name: Procter &Gamble Pharmaceuticals; SIC: 2830;
Name: Alexion Pharmaceuticals Inc; Ticker: ALXN; NAICS: 325412;
Name: Procter &Gamble Co; Ticker: PG; NAICS: 325611, 325612, 325620, 322291, 325412; DUNS: 00-131-6827;

Publication title: PR Newswire

Pagination: 1

Number of pages: 0

Publication year: 2002

Publication date: Mar 8, 2002

Dateline: Connecticut

Publisher: PR Newswire Association LLC

Place of publication: New York

Country of publication: United States

Publication subject: Business And Economics

Source type: Wire Feeds

Language of publication: English

Document type: WIRE FEED

ProQuest document ID: 449103734

Document URL: http://dialog.proquest.com/professional/docview/449103734?accountid=157282

Copyright: Copyright PR Newswire - NY Mar 8, 2002

First available: 2010-06-30

Database: ProQuest Newsstand Professional

Contact ProQuest
Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

Alexion Pharmaceuticals Reports Second Quarter and First Half Results; - Quarter Highlighted by Successful Revision of Pexelizumab Collaboration With Procter &Gamble Pharmaceuticals and Commencement Of Phase III Pivotal PRIMO-CABG Trial -

Publication info: PR Newswire 08 Mar 2002: NA.

ProQuest document link

Abstract (English): CHESHIRE, Conn. -- Alexion Pharmaceuticals, Inc. today announced financial results for its second fiscal quarter and half year ended January 31, 2002.

In its second quarter ended January 31, 2002, Alexion received revenues amounting to $3.38 million compared to $3.17 million received in the same period ended January 31, 2001. Revenues were slightly higher than in the same period last year primarily due to receipt of a $2 million milestone payment from our pexelizumab development partner, Procter &Gamble Pharmaceuticals (P&G) for the initiation of our pivotal Phase III clinical trial in CABG patients, called "PRIMO-CABG."

The Company's research and development expenses for the three month period were $15.04 million compared to $9.40 million for the same period last year.

Full text: CHESHIRE, Conn. -- Alexion Pharmaceuticals, Inc. today announced financial results for its second fiscal quarter and half year ended January 31, 2002.

In its second quarter ended January 31, 2002, Alexion received revenues amounting to $3.38 million compared to $3.17 million received in the same period ended January 31, 2001. Revenues were slightly higher than in the same period last year primarily due to receipt of a $2 million milestone payment from our pexelizumab development partner, Procter &Gamble Pharmaceuticals (P&G) for the initiation of our pivotal Phase III clinical trial in CABG patients, called "PRIMO-CABG."

The Company's research and development expenses for the three month period were $15.04 million compared to $9.40 million for the same period last year. The $5.64 million increase resulted principally from greater manufacturing costs associated with our lead C5 inhibitor candidates, pexelizumab and eculizumab (formerly 5G1.1), and our incurred ongoing pexelizumab Phase III CABG clinical trial costs. As part of the revised collaboration agreement with P&G signed in December 2001, it was agreed that the Company would bear the first 50% of the projected clinical trial costs for the on-going Phase III PRIMO-CABG study and P&G would bear the second half. As such, in the quarter, we recognized a one-time $3.10 million expense for unbilled reimbursable contract costs associated with manufacturing of pexelizumab for Phase III trials which would have been paid by P&G under the original agreement.

General and administrative expenses were $1.84 million for the quarter compared to $1.97 million for the same period last year, mostly as a result of decreased recruiting costs. Accordingly, total operating expenses increased to $16.88 million for the quarter compared to $11.37 million in the three month period ended January 31, 2001, excluding the non-cash amortization of goodwill. The Company posted interest income for the quarter of $3.92 million compared to $6.54 million for the same period last year. The decrease was primarily due to reduced market interest rates. Interest expense, primarily on the Company's $120 million convertible subordinated notes, was $1.93 million for the quarter versus $1.95 million for the same period last year. A state income tax credit was recognized for the three months ended January 31, 2002 representing a state tax benefit of approximately $700,000.

As a result of the above factors, the Company incurred a net loss for the quarter of $10.81 million, or $0.60 per share versus a net loss of $3.61 million or $0.20 per common share for the same three month period in 2001. Taking into account the non-cash amortization of goodwill related to the Company's acquisition of Prolifaron in September 2000, the Company's total net loss amounted to $4.49 million or $0.25 per common share for the quarter ended January 31, 2001.

For the six months ended January 31, 2002, Alexion's revenues were $5.24 million compared to $6.57 million for the same period last year. The $1.33 million decrease in contract research revenues occurred, despite receipt of the $2 million milestone payment, primarily because clinical trial and clinical manufacturing payments from P&G were lower resulting from last year's completion of the Phase IIb pexelizumab CABG study.
Research and development expenditures were $24.71 million for the first half of this fiscal year compared to $20.32 million for the six months ended January 31, 2001. The increase was, again, attributable to higher clinical manufacturing costs associated with our lead C5 inhibitor candidates, pexelizumab and eculizumab, and incurred clinical trial costs for the Company's ongoing pexelizumab Phase III CABG trial.
General and administrative expenses increased slightly to $3.44 million for the six months from $3.35 million for the same period last year mostly as a result of higher payroll costs from added personnel. Accordingly, total operating expenses increased to $28.15 million for the half year compared to $23.67 million, without taking into account last year's acquisition related non-cash charges, in the six month period ended January 31, 2001.
The Company posted interest income for the past six months of $7.46 million compared to $9.34 million for the same period last year. The decrease was primarily due to reduced market interest rates. Interest expense was $3.85 million for the quarter versus $3.95 million for the same period last year. A state income tax credit was recognized in the second quarter ended January 31, 2002 representing a state tax benefit of approximately $700,000.
Net loss for the six months increased to $18.60 million or $1.03 per share from a net loss of $11.70 million or $0.70 per share recorded for the same six month period last year, exclusive of the impact of non-cash charges. Last year's non-cash charges related to Alexion's acquisition of Prolifaron in September 2000 in which the Company recognized a $21.0 million one-time first quarter charge allocated to in-process research and development projects and $1.23 million charge for amortization of goodwill. In addition, the Company recorded a $9.12 million non-cash charge for the cumulative effect of adoption of SAB 101. Taking into account these non-cash charges, the Company's total net loss including operating expenses amounted to $43.05 million or $2.58 per common share for the six months ended January 31, 2001.
As of January 31, 2002 Alexion had approximately $341 million in cash, cash equivalents and marketable securities.
"Alexion achieved several very significant milestones in the second quarter, including the initiation of the pexelizumab pivotal Phase III PRIMO- CABG trial, completion of patient enrollment in the first of our two acute myocardial infarction Phase II trials with pexelizumab, and the successful restructuring of our collaboration agreement with P&G. We are excited that the revised collaboration with P&G allows Alexion to share in decision-making and development and commercial responsibilities for this important product and provides that we and P&G will share the gross margin on sales in the U.S., on an approximately equal basis," said David Keiser, Executive VP and Chief Operating Officer of Alexion. "Our pipeline has made tremendous progress, also in other areas during the quarter, most noteworthy, being the initiation of a Phase IIb trial of eculizumab, formerly 5G1.1, in rheumatoid arthritis patients."
As a result of the revised collaboration with P&G, of which terms include an approximate 50% share of gross margin on U.S. sales and a royalty on sales in the rest of the world, Alexion will incur approximately 50% of all Phase III clinical trial, product development, manufacturing, and commercialization costs necessary for the potential approval and marketing of pexelizumab in the U.S. Additionally, as part of the agreement, Alexion will generally forgo further research and development support payments from P&G. As a result, Alexion anticipates its contract research revenues will not exceed $7.0 million for the current fiscal year, and operating expenses will increase significantly going forward, reaching $78 to $82 million by the end of our fiscal year in July 31, 2002. Net loss for the year ending July 31, 2002 is expected to be in the $65 to $70 million range.
Alexion is engaged in the discovery and development of therapeutic products aimed at treating patients with a wide array of severe disease states, including cardiovascular and autoimmune disorders, inflammation and

cancer. Alexion's two lead product candidates, pexelizumab and eculizumab, are currently in eight clinical development programs. Alexion is developing pexelizumab, an antibody fragment, in collaboration with Procter &Gamble Pharmaceuticals. Together the firms have initiated a Phase III clinical study with pexelizumab in cardiopulmonary bypass patients, and are currently conducting two large Phase II studies with pexelizumab in acute myocardial infarction patients. Alexion's other lead product candidate, eculizumab, is being studied in a Phase IIb trial for the treatment of rheumatoid arthritis. Eculizumab is also in Phase II trials for the treatment of membranous nephritis and for lupus nephritis, and in earlier stage clinical trials for the treatment of dermatomyositis and pemphigoid. Additionally, through its wholly owned subsidiary, Alexion Antibody Technologies, Inc., Alexion is engaged in discovering and developing a portfolio of additional antibody therapeutics targeting severe unmet medical needs. This press release and further information about Alexion Pharmaceuticals, Inc. can be found on the World Wide Web at: http://www.alexionpharm.com/.

This news release contains forward-looking statements. Such statements are subject to certain factors which may cause Alexion's plans to differ or results to vary from those expected, including the results of pre-clinical or clinical studies (including termination or delay in clinical programs or inability to move forward to the next Phase of clinical development), the need for additional research and testing, delays in developing or arranging satisfactory manufacturing capability, inability to access capital and funding on a timely basis and on favorable terms, delays in development of or adverse changes in status of commercial relationships, the possibility that favorable results of earlier clinical trials are not predictive of safety and efficacy results in larger clinical trials, dependence on Procter &Gamble Pharmaceuticals for performance of development and commercial matters related to pexelizumab, the risk that third parties won't agree to license us on reasonable terms their intellectual property necessary for us to develop and commercialize our products, and a variety of other risks set forth from time to time in Alexion's filings with the Securities and Exchange Commission, including but not limited to the risks discussed in Alexion's Annual Report on Form 10-K for the year ended July 31, 2001. Except in special circumstances in which a duty to update arises under law when prior disclosure becomes materially misleading in light of subsequent events, Alexion does not intend to update any of these forward-looking statements to reflect events or circumstances after the date hereof or to reflect the occurrence of unanticipated events.

ALEXION PHARMACEUTICALS, INC.

Selected Financial Data

Statements of Operations

(dollars in thousands except per share amounts)

	Quarter ended January 31, 2002	Quarter ended January 31, 2001	
			Pro forma -a)
REVENUES	$3,380	$3,174	$3,174
OPERATING EXPENSES:			
Research and Development	15,043	9,399	9,399
General and Administrative	1,836	1,971	1,971
In-Process Research and Development (IPRD)	--	--	--
Amortization of Goodwill	--	--	878
Total Operating Expenses	16,879	11,370	12,248
OPERATING INCOME (LOSS)	(13,499)	(8,196)	(9,074)
Interest Income	3,918	6,536	6,536
Interest Expense	(1,929)	(1,948)	(1,948)
State Tax Benefit	700	--	--

NET INCOME (LOSS) $(10,810) $(3,608) -a) $(4,486)
BASIC AND DILUTED NET INCOME
(LOSS) PER COMMON SHARE $(0.60) $(0.20) -a) $(0.25)
SHARES USED IN COMPUTING BASIC
AND DILUTED NET INCOME (LOSS)
PER COMMON SHARE 18,119 17,999 17,999
-a) excludes non-cash IPRD and Amortization of Goodwill
ALEXION PHARMACEUTICALS, INC.
Selected Financial Data
Statements of Operations
(dollars in thousands except per share amounts)
Six Months ended Six Months ended
January 31, January 31,
2002 2001 2001
Pro forma -a)
REVENUES $5,240 $6,573 $6,573
OPERATING EXPENSES:
Research and Development 24,714 20,322 20,322
General and Administrative 3,435 3,349 3,349
In-Process Research
and Development (IPRD) -- -- 21,000
Amortization of Goodwill -- -- 1,227
Total Operating Expenses 28,149 23,671 45,898
OPERATING INCOME (LOSS) (22,909) (17,098) (39,325)
Interest Income 7,456 9,344 9,344
Interest Expense (3,846) (3,946) (3,946)
State Tax Benefit 700 -- --
Cumulative Effect of
Adoption of SAB 101 -- -- (9,118)
NET INCOME (LOSS) $(18,599) $(11,700) -a) $(43,045)
BASIC AND DILUTED NET INCOME
(LOSS) PER COMMON SHARE $(1.03) $(0.70) -a) $(2.58)
SHARES USED IN COMPUTING BASIC AND
DILUTED NET INCOME (LOSS) PER
COMMON SHARE 18,114 16,661 16,661
- a) excludes non-cash IPRD, Amortization of Goodwill, and Cumulative
Effect of Adoption of SAB 101
Balance Sheet Data
(dollars in thousands)
January 31, January 31, July 31,
2002 2001 2001
(unaudited) (unaudited) (audited)
Cash, cash equivalents and
marketable securities $340,939 $369,904 $355,274
Total Assets $383,515 $414,515 $400,259
Net Stockholders' Equity $242,368 $282,214 $260,408

Contacts: Alexion Pharmaceuticals, Inc.
David Keiser
Executive Vice President &COO
(203) 272-2596
Noonan/Russo Communications, Inc. Rx Communications
Ernie Knewitz (Media) Rhonda Chiger (Investor)
(212) 696-4455 Ext. 204 917-322-2569
MAKE YOUR OPINION COUNT - Click Here
http://tbutton.prnewswire.com/prn/11690X78322878

Contact: David Keiser, Executive Vice President &COO of Alexion Pharmaceuticals, Inc., +1-203-272-2596; Media - Ernie Knewitz of Noonan-Russo Communications, Inc., +1-212-696-4455, ext. 204; Investors - Rhonda Chiger of Rx Communications, +1-917-322-2569, both for Alexion Pharmaceuticals, Inc.
Website: http://www.alexionpharm.com/

Subject: Drugs;Pharmaceutical industry

Company / organization: Alexion Pharmaceuticals Inc.; Procter &Gamble Pharmaceuticals

Identifier (keyword): Trade, ALXN

NAICS classification: 325412: Pharmaceutical Preparation Manufacturing

Product classification: 2834000: Pharmaceutical Preparations

SIC classification: 2834: Pharmaceutical preparations

Publication title: PR Newswire

Pagination: NA

Publication date: Mar 8, 2002

Publisher: PR Newswire Association LLC

Journal subject: Business, News, opinion and commentary

Journal code: 4PRN

Source type: Newswire

Language of publication: English

Document type: Magazine/Journal

Source attribution: Gale PROMT, © Publisher specific

Accession number: 83576084

Document URL: http://dialog.proquest.com/professional/docview/678356161?accountid=157282

Copyright: Copyright 2002 Gale, Cengage Learning. All rights reserved.COPYRIGHT 2002 PR Newswire Association LLC

Date created: 2011-08-02

First available: 2010-07-25

Database: Gale Group PROMT® (1972 - current)

Contact ProQuest
Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

Alexion Pharmaceuticals, Inc.; Company reports second quarter, first- half results

Publication info: Biotech Week (Apr 10, 2002): 9.
ProQuest document link

Abstract (summary): "Alexion achieved several very significant milestones in the second quarter, including the initiation of the pexelizumab pivotal phase III PRIMO-CABG trial, completion of patient enrollment in the first of our two acute myocardial infarction phase II trials with pexelizumab, and the successful restructuring of our collaboration agreement with P &G.

Full text: Alexion Pharmaceuticals, Inc., (ALXN) announced financial results for its second fiscal quarter and half year ended January 31, 2002.

In its second quarter ended January 31, 2002, Alexion received revenues amounting to $3.38 million compared with $3.17 million received in the same period ended January 31, 2001. Revenues were slightly higher than in the same period last year primarily due to receipt of a $2 million milestone payment from our pexelizumab development partner, Procter &Gamble Pharmaceuticals (P &G) for the initiation of our pivotal phase III clinical trial in CABG patients, called "PRIMO-CABG."

The company's research and development expenses for the 3-month period were $15.04 million compared with $9.40 million for the same period last year. The $5.64 million increase resulted principally from greater manufacturing costs associated with our lead C5 inhibitor candidates, pexelizumab and eculizumab (formerly 5G1.1), and our incurred ongoing pexelizumab phase III CABG clinical trial costs. As part of the revised collaboration agreement with P &G signed in December 2001, it was agreed that the company would bear the first 50% of the projected clinical trial costs for the on- going phase III PRIMO-CABG study and P &G would bear the second half. As such, in the quarter, we recognized a one-time $3.10 million expense for unbilled reimbursable contract costs associated with manufacturing of pexelizumab for phase III trials which would have been paid by P &G under the original agreement.

General and administrative expenses were $1.84 million for the quarter compared with $1.97 million for the same period last year, mostly as a result of decreased recruiting costs. Accordingly, total operating expenses increased to $16.88 million for the quarter compared with $11.37 million in the three-month period ended January 31, 2001, excluding the noncash amortization of goodwill.

As a result of the above factors, the company incurred a net loss for the quarter of $10.81 million, or $0.60 per share versus a net loss of $3.61 million or $0.20 per common share for the same 3- month period in 2001. Taking into account the noncash amortization of goodwill related to the company's acquisition of Prolifaron in September 2000, the company's total net loss amounted to $4.49 million or $0.25 per common share for the quarter ended January 31, 2001.

For the 6 months ended January 31, 2002, Alexion's revenues were $5.24 million compared with $6.57 million for the same period last year. The $1.33 million decrease in contract research revenues occurred, despite receipt of the $2 million milestone payment, primarily because clinical trial and clinical manufacturing payments from P &G were lower resulting from last year's completion of the phase IIb pexelizumab CABG study.

Research and development expenditures were $24.71 million for the first half of this fiscal year compared with $20.32 million for the 6 months ended January 31, 2001. The increase was, again, attributable to higher clinical manufacturing costs associated with our lead C5 inhibitor candidates, pexelizumab and eculizumab, and incurred clinical trial costs for the company's ongoing pexelizumab phase III CABG trial.

Net loss for the 6 months increased to $18.60 million or $1.03 per share from a net loss of $11.70 million or $0.70 per share recorded for the same 6-month period last year, exclusive of the impact of noncash charges.

"Alexion achieved several very significant milestones in the second quarter, including the initiation of the pexelizumab pivotal phase III PRIMO-CABG trial, completion of patient enrollment in the first of our two acute

myocardial infarction phase II trials with pexelizumab, and the successful restructuring of our collaboration agreement with P &G. We are excited that the revised collaboration with P &G allows Alexion to share in decision-making and development and commercial responsibilities for this important product and provides that we and P &G will share the gross margin on sales in the U.S., on an approximately equal basis," said David Keiser, executive VP and chief operating officer of Alexion. "Our pipeline has made tremendous progress, also in other areas during the quarter, most noteworthy, being the initiation of a phase IIb trial of eculizumab, formerly 5G1.1, in rheumatoid arthritis patients."
This article was prepared by Biotech Week editors from staff and other reports.

Company / organization: Company / organization Name: Alexion Pharmaceuticals Inc; Ticker: ALXN; NAICS: 325412;

Publication title: Biotech Week

Pagination: 9

Number of pages: 0

Publication year: 2002

Publication date: Apr 10, 2002

Publisher: NewsRx

Place of publication: Atlanta

Country of publication: United States

Publication subject: Medical Sciences--Computer Applications

ISSN: 15352757

Source type: Trade Journals

Language of publication: English

Document type: Expanded Reporting

ProQuest document ID: 205521890

Document URL: http://dialog.proquest.com/professional/docview/205521890?accountid=157282

Copyright: Copyright 2002, Biotech Week via NewsRx.com &NewsRx.net

First available: 2010-06-07

Database: ProQuest Newsstand Professional

Contact ProQuest
Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

eculizumab Alexion clinical data (phase III) (PNH)

Publication info: R & D Focus Drug News (Feb 6, 2006): NA.
ProQuest document link

Abstract (English): On 26 January 2006 Alexion reported data from a phase III trial of eculizumab (SOLIRIS), a humanized monoclonal antibody targeted to complement C5, in the treatment of paroxysmal nocturnal hemoglobinuria (PNH). The double-blind, randomized,
placebo-controlled, multicenter phase III trial, designated TRIUMPH, was conducted in the USA, Canada, Europe and Australia. The study evaluated the effects of eculizumab on hemoglobin stabilization and blood transfusion in hemolytic,
transfusion-dependent PNH patients during six months of therapy. The primary endpoints were median transfusion rate and hemoglobin stabilization over six months. Results showed that the median transfusion rate was reduced from 10 units/patient for
placebo, to 0 units/patients for eculizumab (p is less than 0.000000001).

Full text: On 26 January 2006 Alexion reported data from a phase III trial of eculizumab (SOLIRIS), a humanized monoclonal antibody targeted to complement C5, in the treatment of paroxysmal nocturnal hemoglobinuria (PNH). The double-blind, randomized,
placebo-controlled, multicenter phase III trial, designated TRIUMPH, was conducted in the USA, Canada, Europe and Australia. The study evaluated the effects of eculizumab on hemoglobin stabilization and blood transfusion in hemolytic,
transfusion-dependent PNH patients during six months of therapy. The primary endpoints were median transfusion rate and hemoglobin stabilization over six months. Results showed that the median transfusion rate was reduced from 10 units/patient for
placebo, to 0 units/patients for eculizumab (p is less than 0.000000001). Hemoglobin stabilization was achieved by 49% of eculizumab-treated patients compared with 0% of placebo-treated patients (p is less than 0.0000001). European and US regulatory
submissions for eculizumab in the treatment of PNH are planned for the second half 2006.
eculizumab, MAb, C5, MAb, complement C5, h5G1.1, h5G1.1VHC+h5G1.1VLC, SOLIRIS, M1A1, Antirheumatic Non-Steroidal Plain, L4A, Immunosuppressive Agents, D5B, Systemic Antipsoriasis and Similar Products, M5X, Other Musculoskeletal Products, G4B9, All
Other Urological Products, D11A, Other Dermatologicals, B3X, Other Antianemic Products, Alexion, clinical data (phase III)

Subject: Clinical trials;Rheumatoid arthritis

Identifier (keyword): Trade

Publication title: R &D Focus Drug News

Pagination: NA

Publication date: Feb 6, 2006

Publisher: IMSworld Publications Ltd.

Journal subject: Business, Pharmaceuticals and cosmetics industries

ISSN: 1350-1135

Journal code: 0FQN

Source type: Magazine/Journal

Language of publication: English

Document type: Magazine/Journal

Source attribution: Gale PROMT, © Publisher specific

Accession number: 141611385

Document URL: http://dialog.proquest.com/professional/docview/675989538?accountid=157282

Copyright: Copyright 2006 Gale, Cengage Learning. All rights reserved.COPYRIGHT 2006 IMSworld Publications Ltd.

Date created: 2010-04-28

First available: 2010-07-25

Database: Gale Group PROMT® (1972 - current)

Contact ProQuest

Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

eculizumab Alexion clinical data (PNH)

Publication info: R & D Focus Drug News (Jan 30, 2006): NA.

ProQuest document link

Abstract (English): On 12 December 2005, Alexion reported an analysis of three-year cumulative data from an open-label extension study of eculizumab in ten patients with paroxysmal nocturnal hemoglobinuria (PNH). Results showed continued treatment with eculizumab
maintained the reduction in intravascular hemolysis observed in a 12-week pilot study. A sustained and stable increase in PNH red blood cell counts (from 1.37 x10(12)/L to 2.37 x10(12)/L at maximum response) was also seen following three-year chronic
treatment with the agent. The levels of lactate dehydrogenase decreased from a mean of 3233 (+/- 648) during the 12 months before treatment to 656 (+/-37) following eculizumab treatment for at least 36 months (p=0.004). The increase in PNH red blood cells

Full text: On 12 December 2005, Alexion reported an analysis of three-year cumulative data from an open-label extension study of eculizumab in ten patients with paroxysmal nocturnal hemoglobinuria (PNH). Results showed continued treatment with eculizumab
maintained the reduction in intravascular hemolysis observed in a 12-week pilot study. A sustained and stable increase in PNH red blood cell counts (from 1.37 x10(12)/L to 2.37 x10(12)/L at maximum response) was also seen following three-year chronic
treatment with the agent. The levels of lactate dehydrogenase decreased from a mean of 3233 (+/- 648) during the 12 months before treatment to 656 (+/-37) following eculizumab treatment for at least 36 months (p=0.004). The increase in PNH red blood cells
coincided with reductions in the mean and median transfusion rates from 2.1 and 1.8 units/patient/month during the 12 months prior to treatment, to 0.3 and 0.3 units/patient/month (p=0.002) following 36-month eculizumab therapy, respectively. Treatment
with eculizumab alone or in combination with erythropoietin in aplastic patients resulted in seven of the ten patients becoming transfusion independent for at least the last 17 months of the study. Eculizumab was well tolerated; the most common adverse
events reported were headaches, upper respiratory tract infections and nausea. ....Alexion expects to report top line results from a double-blind, randomized, placebo-controlled multicenter phase III trial, designated TRIUMPH, in the first quarter 2006.
The trial evaluated the effects of eculizumab on hemoglobin stabilization and blood transfusion in approximately 75 hemolytic, transfusion-dependent PNH patients during six months of therapy. Eculizumab, a humanized monoclonal antibody targeted to
complement C5, is also undergoing an open-label, non-placebo-controlled, multicenter phase III safety study in hemolytic PNH patients with a history of transfusion.
eculizumab, MAb, C5, MAb, complement C5, h5G1.1, h5G1.1VHC+h5G1.1VLC, M1A1, Antirheumatic Non-Steroidal Plain, L4A, Immunosuppressive Agents, D5B, Systemic Antipsoriasis and Similar Products, M5X, Other Musculoskeletal Products, G4B9, All Other
Urological Products, D11A, Other Dermatologicals, B3X, Other Antianemic Products, Alexion, clinical-data

Subject: Rheumatoid arthritis

Identifier (keyword): Trade

Publication title: R &D Focus Drug News

Pagination: NA

Publication date: Jan 30, 2006

Publisher: IMSworld Publications Ltd.

Journal subject: Business, Pharmaceuticals and cosmetics industries

ISSN: 1350-1135

Journal code: 0FQN

Source type: Magazine/Journal

Language of publication: English

Document type: Magazine/Journal

Source attribution: Gale Health and Wellness, © Publisher specific

Accession number: 141353513

Document URL: http://dialog.proquest.com/professional/docview/769908150?accountid=157282

Copyright: Copyright 2006 Gale, Cengage Learning. All rights reserved.COPYRIGHT 2006 IMSworld Publications Ltd.

Date created: 2010-05-20

First available: 2010-11-15

Database: Gale Group Health Periodicals Database (1983 - current)

Contact ProQuest

Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

eculizumab Alexion completes enrollment for phase III PNH trial

Publication info: R & D Focus Drug News (Apr 25, 2005): NA.

ProQuest document link

Abstract (English): Alexion announced on 12 April 2005 that it has completed enrollment of more than 75 patients for its phase III TRIUMPH trial of eculizumab in patients with paroxysmal nocturnal hemoglobinuria (PNH). The trial aims to evaluate the effects of eculizumab
on hemoglobin stabilization and blood transfusion in transfusion-dependent PNH patients during six months of therapy. All patients are expected to have completed the six-month treatment phase by the end of 2005. In addition, a second phase III trial,
designated SHEPHERD, is under way to provide additional safety data for eculizumab in hemolytic PNH patients with a history of transfusion. ....Eculizumab, a humanized monoclonal antibody targeted to complement C5, may also have potential in the treatment

Full text: Alexion announced on 12 April 2005 that it has completed enrollment of more than 75 patients for its phase III TRIUMPH trial of eculizumab in patients with paroxysmal nocturnal hemoglobinuria (PNH). The trial aims to evaluate the effects of eculizumab
on hemoglobin stabilization and blood transfusion in transfusion-dependent PNH patients during six months of therapy. All patients are expected to have completed the six-month treatment phase by the end of 2005. In addition, a second phase III trial,
designated SHEPHERD, is under way to provide additional safety data for eculizumab in hemolytic PNH patients with a history of transfusion. ....Eculizumab, a humanized monoclonal antibody targeted to complement C5, may also have potential in the treatment
of autoimmune diseases such as rheumatoid arthritis and other inflammatory disorders.
eculizumab, MAb, C5, MAb, complement C5, h5G1.1, h5G1.1VHC+h5G1.1VLC, M1A1, Antirheumatic Non-Steroidal Plain, L4A, Immunosuppressive Agents, D5A, Topical Antipsoriasis and Similar Products, M5X, Other Musculoskeletal Products, G4B9, All Other
Urological Products, D11A, Other Dermatologicals, B3X, Other Antianemic Products, Alexion

Subject: Clinical trials;Pharmaceutical industry -- Product development;Product development

Location: United States

Company / organization: Alexion Pharmaceuticals Inc.; Alexion Pharmaceuticals Inc. (Product development)

Identifier (keyword): Trade, ALXN

Event classification: 331: Product development

SIC classification: 2834: Pharmaceutical preparations

Publication title: R &D Focus Drug News

Pagination: NA

Publication date: Apr 25, 2005

Publisher: IMSworld Publications Ltd.

Journal subject: Business, Pharmaceuticals and cosmetics industries

ISSN: 1350-1135

Journal code: 0FQN

Source type: Magazine/Journal

Language of publication: English

Document type: Brief Article

Source attribution: Gale Health and Wellness, © Publisher specific

Accession number: 131849209

Document URL: http://dialog.proquest.com/professional/docview/770906683?accountid=157282

Copyright: Copyright 2005 Gale, Cengage Learning. All rights reserved.COPYRIGHT 2005 IMSworld Publications Ltd.

Date created: 2010-05-20

First available: 2010-11-15

Database: Gale Group Health Periodicals Database (1983 - current)

Contact ProQuest

Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

eculizumab Alexion completes enrollment

Publication info: R & D Focus Drug News (Feb 17, 2003): NA.
ProQuest document link

Abstract (English): Alexion has completed enrollment in a phase IIb trial of eculizumab, its humanized monoclonal antibody targeting complement C5, in the treatment of rheumatoid arthritis. Approximately 350 patients with rheumatoid arthritis are enrolled in the study,
which will assess safety and efficacy of the monoclonal, and will confirm an effective dosing regimen. In the randomized, double-blind, placebo-controlled trial, patients are to receive either placebo, iv eculizumab once weekly for four weeks then once
monthly, or iv eculizumab once weekly for four weeks followed by once every two weeks. After six months, patients are to be evaluated for safety and efficacy; the primary endpoint is improvement in ACR 20 score.

Full text: Alexion has completed enrollment in a phase IIb trial of eculizumab, its humanized monoclonal antibody targeting complement C5, in the treatment of rheumatoid arthritis. Approximately 350 patients with rheumatoid arthritis are enrolled in the study,
which will assess safety and efficacy of the monoclonal, and will confirm an effective dosing regimen. In the randomized, double-blind, placebo-controlled trial, patients are to receive either placebo, iv eculizumab once weekly for four weeks then once
monthly, or iv eculizumab once weekly for four weeks followed by once every two weeks. After six months, patients are to be evaluated for safety and efficacy; the primary endpoint is improvement in ACR 20 score.
eculizumab, MAb, C5, MAb, complement C5, h5G1.1, h5G1.1VHC+h5G1.1VLC, M1A1, Antirheumatic Non-Steroidal Plain, L4A, Immunosuppressive Agents, D5, Nonsteroidal Products for Inflammatory Skin Disorders Including Psoriasis, M5X, Other Musculoskeletal
Products, G4B9, All Other Urological Products, D11A, Other Dermatologicals, Alexion

Subject: Care and treatment;Monoclonal antibodies -- Product development;Patient care;Patients;Patients -- Care and treatment;Pharmaceutical industry -- Product development;Product development;Rheumatoid arthritis -- Care and treatment

Location: United States

Company / organization: Alexion Pharmaceuticals Inc.; Alexion Pharmaceuticals Inc. (Product development)

Identifier (keyword): Trade, ALXN

Event classification: 331: Product development

Alternate title: in a phase IIb trial

Publication title: R &D Focus Drug News

Pagination: NA

Publication date: Feb 17, 2003

Publisher: IMSworld Publications Ltd.

Journal subject: Business, Pharmaceuticals and cosmetics industries

ISSN: 1350-1135

Journal code: 0FQN

Source type: Magazine/Journal

Language of publication: English

Document type: Brief Article

Source attribution: Gale Health and Wellness, © Publisher specific

Accession number: 97619207

Document URL: http://dialog.proquest.com/professional/docview/770534280?accountid=157282

Copyright: Copyright 2003 Gale, Cengage Learning. All rights reserved.COPYRIGHT 2003 IMSworld Publications Ltd.

Date created: 2010-05-20

First available: 2010-11-15

Database: Gale Group Health Periodicals Database (1983 - current)

Contact ProQuest

Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

eculizumab Alexion EMEA grants request for accelerated assessment procedure

Publication info: R & D Focus Drug News (Aug 28, 2006): NA.
ProQuest document link

Abstract (English): On 8 August 2006 Alexion announced that the EMEA has accepted its request to evaluate an MAA for eculizumab (SOLIRIS) in the treatment of nocturnal hemoglobinuria (PNH), under the accelerated assessment procedure. Eculizumab, a humanized monoclonal
antibody targeted to complement C5, is being developed for the treatment of PNH. Alexion plans to submit a BLA to the US FDA and an MAA to the EMEA for the treatment of PNH second half 2006.
eculizumab, MAb, C5, MAb, complement C5, h5G1.1, h5G1.1VHC+h5G1.1VLC, SOLIRIS, M1C, Specific Antirheumatic Agents, L4A, Immunosuppressive Agents, D5B, Systemic Antipsoriasis and Similar Products, M5X, All Other Musculoskeletal Products, G4B9, All
Other Urological Products, D11A, Other Dermatologicals, B3X, Other Antianemic Products, Alexion

Full text: On 8 August 2006 Alexion announced that the EMEA has accepted its request to evaluate an MAA for eculizumab (SOLIRIS) in the treatment of nocturnal hemoglobinuria (PNH), under the accelerated assessment procedure. Eculizumab, a humanized monoclonal
antibody targeted to complement C5, is being developed for the treatment of PNH. Alexion plans to submit a BLA to the US FDA and an MAA to the EMEA for the treatment of PNH second half 2006.
eculizumab, MAb, C5, MAb, complement C5, h5G1.1, h5G1.1VHC+h5G1.1VLC, SOLIRIS, M1C, Specific Antirheumatic Agents, L4A, Immunosuppressive Agents, D5B, Systemic Antipsoriasis and Similar Products, M5X, All Other Musculoskeletal Products, G4B9, All
Other Urological Products, D11A, Other Dermatologicals, B3X, Other Antianemic Products, Alexion

Subject: Drug therapy;Monoclonal antibodies;Rheumatoid arthritis -- Drug therapy

Identifier (keyword): Trade

Publication title: R &D Focus Drug News

Pagination: NA

Publication date: Aug 28, 2006

Publisher: IMSworld Publications Ltd.

Journal subject: Business, Pharmaceuticals and cosmetics industries

ISSN: 1350-1135

Journal code: 0FQN

Source type: Magazine/Journal

Language of publication: English

Document type: Magazine/Journal

Source attribution: Gale PROMT, © Publisher specific

Accession number: 150062884

Document URL: http://dialog.proquest.com/professional/docview/670473159?accountid=157282

Copyright: Copyright 2006 Gale, Cengage Learning. All rights reserved.COPYRIGHT 2006 IMSworld Publications Ltd.

Date created: 2010-04-28

First available: 2010-07-25

Database: Gale Group PROMT® (1972 - current)

Contact ProQuest

Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

eculizumab Alexion initiates second phase III study

Publication info: R & D Focus Drug News (Jan 17, 2005): NA.
ProQuest document link

Abstract (English): Alexion announced on 10 January 2005 that it has initiated a second phase III trial, designated SHEPHERD, to provide additional safety data for eculizumab in the treatment of paroxysmal nocturnal hemoglobinuria (PNH) in hemolytic patients with a
history of transfusion. The open-label, non-placebo-controlled, multicenter trial aims to enroll up to 75 patients at centers in the USA, Canada, Europe and Australia. Initial results from the trial are expected second half 2005. A phase III trial,
designated TRIUMPH, initiated in November 2004 and is evaluating the effects of eculizumab on hemoglobin stabilization and blood transfusion in approximately 75 hemolytic, transfusion-dependent PNH patients during six months of therapy. The SHEPEHRD and

Full text: Alexion announced on 10 January 2005 that it has initiated a second phase III trial, designated SHEPHERD, to provide additional safety data for eculizumab in the treatment of paroxysmal nocturnal hemoglobinuria (PNH) in hemolytic patients with a
history of transfusion. The open-label, non-placebo-controlled, multicenter trial aims to enroll up to 75 patients at centers in the USA, Canada, Europe and Australia. Initial results from the trial are expected second half 2005. A phase III trial,
designated TRIUMPH, initiated in November 2004 and is evaluating the effects of eculizumab on hemoglobin stabilization and blood transfusion in approximately 75 hemolytic, transfusion-dependent PNH patients during six months of therapy. The SHEPEHRD and
TRIUMPH trials will provide the basis of a BLA filing. Alexion received Orphan Drug designation for eculizumab in the treatment of PNH from the US FDA and the EMEA in December 2003. Eculizumab, a humanized monoclonal antibody targeted to complement C5, is
also being developed for the treatment of idiopathic membranous glomerulonephritis and dermatomyositis.
eculizumab, MAb, C5, MAb, complement C5, h5G1.1, h5G1.1VHC+h5G1.1VLC, M1A1, Antirheumatic Non-Steroidal Plain, L4A, Immunosuppressive Agents, D5A, Topical Antipsoriasis and Similar Products, M5X, Other Musculoskeletal Products, G4B9, All Other
Urological Products, D11A, Other Dermatologicals, B3X, Other Antianemic Products, Alexion

Subject: Antirheumatic agents -- Licensing, certification and accreditation;Licensing, certification and accreditation;Pharmaceutical industry -- Product development;Product development

Location: United States

Company / organization: Alexion Pharmaceuticals Inc.; Alexion Pharmaceuticals Inc. (Product development)

Product name: eculizumab (Medication) (Licensing, certification and accreditation)

Identifier (keyword): Trade, ALXN

Event classification: 331: Product development;930: Government regulation

SIC classification: 2834: Pharmaceutical preparations

Alternate title: Alexion Pharmaceuticals Inc

Publication title: R &D Focus Drug News

Pagination: NA

Publication date: Jan 17, 2005

Publisher: IMSworld Publications Ltd.

Journal subject: Business, Pharmaceuticals and cosmetics industries

ISSN: 1350-1135

Journal code: 0FQN

Source type: Magazine/Journal

Language of publication: English

Document type: Brief Article

Source attribution: Gale Health and Wellness, © Publisher specific

Accession number: 127042468

Document URL: http://dialog.proquest.com/professional/docview/771032150?accountid=157282

Copyright: Copyright 2005 Gale, Cengage Learning. All rights reserved.COPYRIGHT 2005 IMSworld Publications Ltd.

Date created: 2010-05-20

First available: 2010-11-15

Database: Gale Group Health Periodicals Database (1983 - current)

Contact ProQuest

Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

eculizumab Alexion plans phase III trials (paroxysmal nocturnal hemoglobinuria)

Publication info: R & D Focus Drug News (Aug 2, 2004): NA.

ProQuest document link

Abstract (English): Alexion reported that the US FDA has approved its protocol of two phase III trials of eculizumab, a humanized monoclonal antibody targeted to complement C, for the treatment of paroxysmal nocturnal hemoglobinuria (PNH), which are planned to commence
during 2004; the TRIUMPH trial aims to examine the effects of eculizumab on hemoglobin stabilization and blood transfusion in hemolytic transfusion-dependent PNH patients during six months of therapy. The double-blind, randomized, placebo-controlled
multicenter TRIUMPH study aims to enroll approximately 75 patients and will be conducted at sites in the USA, Canada, Europe, Australia and New Zealand. ....A second trial, designated SHEPHERD, aims to provide additional safety data of eculizumab in

Full text: Alexion reported that the US FDA has approved its protocol of two phase III trials of eculizumab, a humanized monoclonal antibody targeted to complement C, for the treatment of paroxysmal nocturnal hemoglobinuria (PNH), which are planned to commence
during 2004; the TRIUMPH trial aims to examine the effects of eculizumab on hemoglobin stabilization and blood transfusion in hemolytic transfusion-dependent PNH patients during six months of therapy. The double-blind, randomized, placebo-controlled
multicenter TRIUMPH study aims to enroll approximately 75 patients and will be conducted at sites in the USA, Canada, Europe, Australia and New Zealand. ....A second trial, designated SHEPHERD, aims to provide additional safety data of eculizumab in
hemolytic PNH patients with a history of transfusion. The SHEPHERD study will be an open-label, nonrandomized, nonplacebo-controlled, multicenter trial and aims to enroll approximately 75 patients at sites in the USA, Canada, Europe, Australia and New
Zealand. ....In December 2003 Alexion received Orphan Drug designation for eculizumab in the treatment of PNH from the US FDA and the EMEA. The compound is also being evaluated for the treatment of autoimmune diseases such as rheumatoid arthritis as well
as musculoskeletal disorders and psoriasis.
eculizumab, MAb, C5, MAb, complement C5, h5G1.1, h5G1.1VHC+h5G1.1VLC, M1A1, Antirheumatic Non-Steroidal Plain, L4A, Immunosuppressive Agents, D5A, Topical Antipsoriasis and Similar Products, M5X, Other Musculoskeletal Products, G4B9, All Other
Urological Products, D11A, Other Dermatologicals, B3X, Other Antianemic Products, Alexion

Subject: Pharmaceutical industry -- Product development;Product development

Location: United States

Company / organization: Alexion Pharmaceuticals Inc.; Alexion Pharmaceuticals Inc. (Product development)

Identifier (keyword): Trade, ALXN

Event classification: 331: Product development

SIC classification: 2834: Pharmaceutical preparations

Publication title: R &D Focus Drug News

Pagination: NA

Publication date: Aug 2, 2004

Publisher: IMSworld Publications Ltd.

Journal subject: Business, Pharmaceuticals and cosmetics industries

ISSN: 1350-1135

Journal code: 0FQN

Source type: Magazine/Journal

Language of publication: English

Document type: Brief Article

Source attribution: Gale PROMT, © Publisher specific

Accession number: 120019526

Document URL: http://dialog.proquest.com/professional/docview/670237236?accountid=157282

Copyright: Copyright 2004 Gale, Cengage Learning. All rights reserved.COPYRIGHT 2004 IMSworld Publications Ltd.

Date created: 2010-04-28

First available: 2010-07-25

Database: Gale Group PROMT® (1972 - current)

Contact ProQuest

Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

eculizumab Alexion Priority Review, USA (PNH)

Publication info: R & D Focus Drug News (Nov 20, 2006): NA.

ProQuest document link

Abstract (English): Alexion announced on 14 November 2006 that the US FDA has accepted and granted Priority Review designation for its Biologics License Application (BLA) seeking approval of eculizumab (SOLIRIS), in the treatment of paroxysmal nocturnal hemoglobinuria
(PNH). Alexion filed the BLA in September 2006. ....Eculizumab was also submitted for approval for this indication in the EU in September 2006 and is undergoing review through the EMEA's Accelerated Assessment Procedure. Eculizumab is a humanized
monoclonal antibody targeted to complement C5, designed to inhibit complement activation with a prolonged duration of action and therefore with potential in limiting inflammation in chronic autoimmune diseases.

Full text: Alexion announced on 14 November 2006 that the US FDA has accepted and granted Priority Review designation for its Biologics License Application (BLA) seeking approval of eculizumab (SOLIRIS), in the treatment of paroxysmal nocturnal hemoglobinuria
(PNH). Alexion filed the BLA in September 2006. ....Eculizumab was also submitted for approval for this indication in the EU in September 2006 and is undergoing review through the EMEA's Accelerated Assessment Procedure. Eculizumab is a humanized
monoclonal antibody targeted to complement C5, designed to inhibit complement activation with a prolonged duration of action and therefore with potential in limiting inflammation in chronic autoimmune diseases.
eculizumab, MAb, C5, MAb, complement C5, h5G1.1, h5G1.1VHC+h5G1.1VLC, SOLIRIS, M1C, Specific Antirheumatic Agents, L4A, Immunosuppressive Agents, D5B, Systemic Antipsoriasis and Similar Products, M5X, All Other Musculoskeletal Products, G4B9, All
Other Urological Products, D11A, Other Dermatologicals, B3X, Other Antianemic Products, Alexion, Priority Review, USA

Subject: Drug therapy;Rheumatoid arthritis -- Drug therapy

Identifier (keyword): Trade

Publication title: R &D Focus Drug News

Pagination: NA

Publication date: Nov 20, 2006

Publisher: IMSworld Publications Ltd.

Journal subject: Business, Pharmaceuticals and cosmetics industries

ISSN: 1350-1135

Journal code: 0FQN

Source type: Magazine/Journal

Language of publication: English

Document type: Magazine/Journal

Source attribution: Gale Health and Wellness, © Publisher specific

Accession number: 154563916

Document URL: http://dialog.proquest.com/professional/docview/770874812?accountid=157282

Copyright: Copyright 2006 Gale, Cengage Learning. All rights reserved.COPYRIGHT 2006 IMSworld Publications Ltd.

Date created: 2010-05-20

First available: 2010-11-15

Database: Gale Group Health Periodicals Database (1983 - current)

Contact ProQuest

Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

Kidney Disease; Enrollment complete for phase II membranous nephritis clinical trial with 5G1.1

Publication info: Drug Week (Apr 12, 2002): 14.
ProQuest document link

Abstract (summary): "Completing enrollment in the first eculizumab phase II trial in patients suffering from membranous nephritis brings us closer to our objective of expanding the profile of eculizumab into a variety of chronic autoimmune opportunities," stated Leonard Bell, MD, president and CEO of Alexion. "Once the 4-month treatment period and all follow-up patient visits are completed, we expect to complete data collection and then lock the database for subsequent analysis of this phase II trial.

Full text: Alexion Pharmaceuticals, Inc., (ALXN) announced that it has completed enrollment in its first phase II trial in patients suffering from a severe form of kidney disease known as membranous nephritis, with its anti-inflammatory C5 inhibitor antibody, 5G1.1, now called eculizumab.

Separately, Alexion announced that it has received orphan drug designation from the U.S. Food and Drug Administration for the use of eculizumab, if approved, in patients with membranous nephritis.

This phase II multicenter, double-blind, randomized, placebo- controlled study examines the safety and clinical efficacy of repeated dosing of eculizumab during a 4-month treatment period. Approximately 115 membranous nephritis patients were enrolled in the United States. Alexion also announced that, as a part of its comprehensive development plan for eculizumab, it has already commenced dosing in a follow-on open label extension study of eculizumab in these patients, designed to examine long-term safety and tolerability.

"Completing enrollment in the first eculizumab phase II trial in patients suffering from membranous nephritis brings us closer to our objective of expanding the profile of eculizumab into a variety of chronic autoimmune opportunities," stated Leonard Bell, MD, president and CEO of Alexion. "Once the 4-month treatment period and all follow-up patient visits are completed, we expect to complete data collection and then lock the database for subsequent analysis of this phase II trial. Additionally, we are encouraged by the orphan drug designation as it may provide an innovative therapy for this small, but underserved, patient population, while potentially providing us with development and commercial advantages."

Membranous nephritis is a form of kidney disease that is believed to be caused by a chronic autoimmune disorder.

This article was prepared by Drug Week editors from staff and other reports.

Company / organization: Company / organization Name: Alexion Pharmaceuticals Inc; Ticker: ALXN; NAICS: 325412;

Publication title: Drug Week

Pagination: 14

Number of pages: 0

Publication year: 2002

Publication date: Apr 12, 2002

Publisher: NewsRx

Place of publication: Atlanta

Country of publication: United States

Publication subject: Medical Sciences

ISSN: 15316440

Source type: Trade Journals

Language of publication: English

Document type: Expanded Reporting

ProQuest document ID: 206266314

Document URL: http://dialog.proquest.com/professional/docview/206266314?accountid=157282

Copyright: Copyright 2002, Drug Week via NewsRx.com &NewsRx.net

First available: 2010-06-07

Database: ProQuest Newsstand Professional

Contact ProQuest
Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

Kidney Disease; Enrollment complete for phase II membranous nephritis clinical trial with 5G1.1

Publication info: Immunotherapy Weekly (Apr 10, 2002): 18.
ProQuest document link

Abstract (summary): "Completing enrollment in the first eculizumab phase II trial in patients suffering from membranous nephritis brings us closer to our objective of expanding the profile of eculizumab into a variety of chronic autoimmune opportunities," stated Leonard Bell, MD, president and CEO of Alexion. "Once the 4-month treatment period and all follow-up patient visits are completed, we expect to complete data collection and then lock the database for subsequent analysis of this phase II trial.

Full text: Alexion Pharmaceuticals, Inc., (ALXN) announced that it has completed enrollment in its first phase II trial in patients suffering from a severe form of kidney disease known as membranous nephritis, with its anti-inflammatory C5 inhibitor antibody, 5G1.1, now called eculizumab.

Separately, Alexion announced that it has received orphan drug designation from the U.S. Food and Drug Administration for the use of eculizumab, if approved, in patients with membranous nephritis.

This phase II multicenter, double-blind, randomized, placebo- controlled study examines the safety and clinical efficacy of repeated dosing of eculizumab during a 4-month treatment period. Approximately 115 membranous nephritis patients were enrolled in the United States. Alexion also announced that, as a part of its comprehensive development plan for eculizumab, it has already commenced dosing in a follow-on open label extension study of eculizumab in these patients, designed to examine long-term safety and tolerability.

"Completing enrollment in the first eculizumab phase II trial in patients suffering from membranous nephritis brings us closer to our objective of expanding the profile of eculizumab into a variety of chronic autoimmune opportunities," stated Leonard Bell, MD, president and CEO of Alexion. "Once the 4-month treatment period and all follow-up patient visits are completed, we expect to complete data collection and then lock the database for subsequent analysis of this phase II trial. Additionally, we are encouraged by the orphan drug designation as it may provide an innovative therapy for this small, but underserved, patient population, while potentially providing us with development and commercial advantages."

Membranous nephritis is a form of kidney disease that is believed to be caused by a chronic autoimmune disorder.

This article was prepared by Immunotherapy Weekly editors from staff and other reports.

Company / organization: Company / organization Name: Alexion Pharmaceuticals Inc; Ticker: ALXN; NAICS: 325412;

Publication title: Immunotherapy Weekly

Pagination: 18

Number of pages: 0

Publication year: 2002

Publication date: Apr 10, 2002

Publisher: NewsRx

Place of publication: Atlanta

Country of publication: United States

Publication subject: Medical Sciences--Allergology And Immunology

ISSN: 10908625

Source type: Trade Journals

Language of publication: English

Document type: Expanded Reporting

ProQuest document ID: 212906164

Document URL: http://dialog.proquest.com/professional/docview/212906164?accountid=157282

Copyright: Copyright 2002, Immunotherapy Weekly via NewsRx.com &NewsRx.net

First available: 2010-06-08

Database: ProQuest Newsstand Professional

Contact ProQuest
Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

eculizumab Alexion phase change III, USA, Europe Canada, Australia (PNH)

Publication info: R & D Focus Drug News (Nov 15, 2004): NA.
ProQuest document link

Abstract (English): Alexion announced on 1 November 2004 that it has initiated a phase III trial to evaluate its humanized monoclonal antibody targeted to complement C5, eculizumab, in the treatment of paroxysmal nocturnal hemoglobinuria (PNH). The double-blind,
randomized, placebo-controlled multicenter trial, designated TRIUMPH, will evaluate the effect of eculizumab on hemoglobin stabilization and blood transfusion in approximately 75 hemolytic, transfusion-dependent PNH patients during six months of therapy.
The trial is being conducted at sites in the USA, Canada, Europe and Australia. ....A second phase III trial, designated SHEPHERD, is also planned for 2004. This trial aims to provide additional safety data for eculizumab in the treatment of hemolytic PNH

Full text: Alexion announced on 1 November 2004 that it has initiated a phase III trial to evaluate its humanized monoclonal antibody targeted to complement C5, eculizumab, in the treatment of paroxysmal nocturnal hemoglobinuria (PNH). The double-blind,
randomized, placebo-controlled multicenter trial, designated TRIUMPH, will evaluate the effect of eculizumab on hemoglobin stabilization and blood transfusion in approximately 75 hemolytic, transfusion-dependent PNH patients during six months of therapy.
The trial is being conducted at sites in the USA, Canada, Europe and Australia. ....A second phase III trial, designated SHEPHERD, is also planned for 2004. This trial aims to provide additional safety data for eculizumab in the treatment of hemolytic PNH
patients with a history of transfusion. ....The US FDA approved the design of both trials in July 2004, under the FDA's Special Protocol Assessment process. Alexion plans to submit a BLA to the US FDA for the use of eculizumab for this indication, based
on results from these trials. The compound is also being evaluated for the treatment of autoimmune diseases, such as rheumatoid arthritis.
eculizumab, MAb, C5, MAb, complement C5, h5G1.1, h5G1.1VHC+h5G1.1VLC, M1A1, Antirheumatic Non-Steroidal Plain, L4A, Immunosuppressive Agents, D5A, Topical Antipsoriasis and Similar Products, M5X, Other Musculoskeletal Products, G4B9, All Other
Urological Products, D11A, Other Dermatologicals, B3X, Other Antianemic Products, Alexion, phase-III, USA, Europe, Canada, Australia, new-phase

Subject: Care and treatment;Clinical trials;Drugs -- Evaluation;Evaluation;Pharmaceutical industry -- Services; Rheumatoid arthritis -- Care and treatment;Services

Location: United States

Company / organization: Alexion Pharmaceuticals Inc.; Alexion Pharmaceuticals Inc. (Services)

Identifier (keyword): Trade, ALXN

Event classification: 360: Services information

NAICS classification: 325412: Pharmaceutical Preparation Manufacturing;3254: Pharmaceutical and Medicine Manufacturing

Product classification: 2834199: Metabolic Agents NEC;2834190: Metabolic Agents;2830000: Drugs &Pharmaceuticals;2834000: Pharmaceutical Preparations

SIC classification: 2834: Pharmaceutical preparations;2830: Drugs

Alternate title: eculizumab Alexion phase change III, USA, Europe, Canada Australia (PNH)

Publication title: R &D Focus Drug News

Pagination: NA

Publication date: Nov 15, 2004

Publisher: IMSworld Publications Ltd.

Journal subject: Business, Pharmaceuticals and cosmetics industries

ISSN: 1350-1135

Journal code: 0FQN

Source type: Magazine/Journal

Language of publication: English

Document type: Brief Article

Source attribution: Gale PROMT, © Publisher specific

Accession number: 124562190

Document URL: http://dialog.proquest.com/professional/docview/671190818?accountid=157282

Copyright: Copyright 2004 Gale, Cengage Learning. All rights reserved.COPYRIGHT 2004 IMSworld Publications Ltd.

Date created: 2010-04-28

First available: 2010-07-25

Database: Gale Group PROMT® (1972 - current)

Contact ProQuest
Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

THERAPEUTIC ANTIBODIES FOR HUMAN DISEASES AT THE DAWN OF THE TWENTY-FIRST CENTURY

Ole Henrik Brekke and Inger Sandlie‡*

Antibodies are highly specific, naturally evolved molecules that recognize and eliminate pathogenic and disease antigens. The past 30 years of antibody research have hinted at the promise of new versatile therapeutic agents to fight cancer, autoimmune diseases and infection. Technology development and the testing of new generations of antibody reagents have altered our view of how they might be used for prophylactic and therapeutic purposes. The therapeutic antibodies of today are genetically engineered molecules that are designed to ensure high specificity and functionality. Some antibodies are loaded with toxic modules, whereas others are designed to function naturally, depending on the therapeutic application. In this review, we discuss various aspects of antibodies that are relevant to their use as as therapeutic agents.

IMMUNOGLOBULIN DOMAIN
Compactly folded globular units of approximately 110 amino acids that comprise immunoglobulin heavy and light chains.

CYTOKINES
A class of small proteins released by one cell that affects the physiology of other cells locally and systemically in a particular fashion through binding to a specific receptor.

**Affitech AS, Gaustadalleen 21, N-0349 Oslo, Norway. ‡Department of Biology, University of Oslo, N-0349, Norway. Correspondence to O.H.B. e-mail: o.h.brekke@affitech.com*
doi:10.1038/nrd984

Antibody structure

The typical antibody — or immunoglobulin (Ig) — consists of two antigen-binding fragments (Fabs), which are linked via a flexible region (the hinge) to a constant (Fc) region (FIG. 1). This structure comprises two pairs of polypeptide chains, each pair containing a heavy and a light chain of different sizes. Both heavy and light chains are folded into IMMUNOGLOBULIN DOMAINS. The 'variable domains' in the amino-terminal part of the molecule are the domains that recognize and bind antigens; the rest of the molecule is composed of 'constant domains' that only vary between Ig classes (BOX 1). The Fc portion of the Ig serves to bind various effector molecules of the immune system, as well as molecules that determine the biodistribution of the antibody.

Mechanisms of *in vivo* action

In antibody-based therapies, the goal is to eliminate or neutralize the pathogenic infection or the disease target, for example, bacterial, viral or tumour targets. Therapeutic antibodies can function by three principal modes of action: by blocking the action of specific molecules, by targeting specific cells or by functioning as signalling molecules. The blocking activity of therapeutic antibodies is achieved by preventing growth factors, CYTOKINES or other soluble mediators reaching their target receptors, which can be accomplished either by the antibody binding to the factor itself or to its receptor. Targeting involves directing antibodies towards specific populations of cells and is a versatile approach; antibodies can be engineered to carry effector moieties, such as enzymes, toxins, radionuclides, cytokines or even DNA molecules, to the target cells, where the attached moiety can then exert its effect (for example, toxins or radionuclides can eliminate target cancer cells). The natural EFFECTOR FUNCTIONS of antibodies are associated with binding to Fc receptors or binding to complement proteins and inducing COMPLEMENT-DEPENDENT CYTOTOXICITY (CDC). Targeting antibodies can retain such effector functions intact or they can be abolished during the design of the antibody, depending on the therapeutic strategy. The signalling effect of antibodies is predicated on either inducing crosslinking of receptors that are, in turn, connected to mediators of cell division or PROGRAMMED CELL DEATH, or directing them towards specific receptors to act as agonists for the

© 2002 Nature Publishing Group

Samsung Bioepis Exhibit No. 1002
Page 910

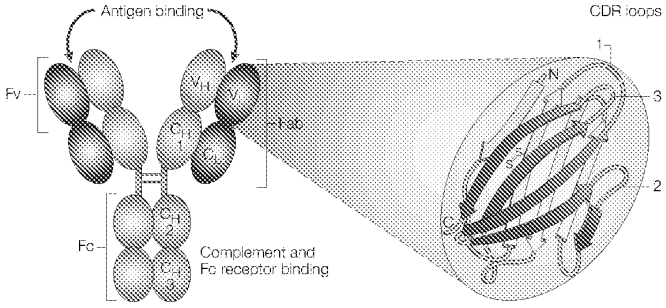


Figure 1 | **The modular structure of immunoglobulins.** This figure shows a single immunoglobulin (Ig) molecule. All immunoglobulin monomers are composed of two identical light (L) chains and two identical heavy (H) chains. Light chains are composed of one constant domain (C_L) and one variable domain (V_L), whereas heavy chains are composed of three constant domains (C_H1, C_H2 and C_H3) and one variable domain (V_H). The heavy chains are covalently linked in the hinge region and the light chains are covalently linked to the heavy chain. The variable domains of both the heavy and light chains compose the antigen-binding part of the molecule, termed Fv. Within the variable domains there are three loops designated complementarity-determining regions (CDRs) 1, 2 and 3, which confer the highest diversity and define the specificity of antibody binding. The Fc portion is glycosylated and contains the sites for interaction with effector molecules, such as the C1 complex of the complement system and a variety of Fc receptors including the neonatal Fc receptor (FcRn).

activation of specific cell populations. Another approach is to use antibodies as delivery vehicles for DNA and, more recently, to deliver antigens to certain immune cells that present processed antigenic peptides, or epitopes, to T cells, to activate a specific immune response against that antigen.

The development of therapeutic antibodies

Target specificity in the treatment and prophylaxis of diseases such as infection, cancer and autoimmune disorders has become more viable through the development of monoclonal antibodies. The mouse HYBRIDOMA technology described by Köhler and Milstein was an important step in the development of antibody technology and paved the way for the emergence of therapeutic monoclonal antibodies[1]. During the 1980s, resources were directed towards the evaluation of the *in vivo* use of mouse monoclonal antibodies in humans, aimed at both imaging and therapy[2]. Mouse monoclonal antibodies were shown to have limited use as therapeutic agents because of a short serum half-life, an inability to trigger human effector functions and the production of human antimouse-antibodies[3] (the HAMA response). In an attempt to reduce the immunogenicity of mouse antibodies, genetic engineering was used to generate chimeric antibodies, that is, antibodies with human constant regions and mouse variable regions[4,5]. However, although chimeric antibodies were perceived as less foreign, and therefore less immunogenic, than mouse monoclonal antibodies, human anti-chimeric antibody responses (HACAs) have nonetheless been observed[6]. Further minimization of the mouse component of antibodies was achieved through CDR (complementarity-determining region) grafting[7] (FIG. 2). In such 'humanized' antibodies, only the CDR loops that are responsible for antigen binding are inserted into the human variable-domain framework. The ability to manipulate antibodies into more human variants finally made antibodies useful for clinical use. With the isolation of genes encoding human variable regions, their successful expression in *Escherichia coli*[8,9] and the introduction of phage-display technology[10], the task of selecting fully human variable domains has been greatly simplified.

Selection from phage-display libraries of human antibody fragments is today the most used and well-established technology for the development of new human antibodies (FIG. 3a). Another approach is to use mice that are transgenic for the human Ig locus[11]. Immunization of such a transgenic mouse results in a human antibody response, from which hybridomas that produce human antibodies can be generated (FIG. 3b).

Today there are approximately 200 antibodies in clinical trials and the US Food and Drug Administration has approved several antibodies against cancer[12,13], transplant rejection[14], rheumatoid arthritis and Crohn's disease[15,16], and antiviral prophylaxis[17] (TABLE 1). So far, 20% of all biopharmaceuticals in clinical trials are monoclonal antibodies (examples are shown in TABLE 2, and see clinical trials web site in online links box), making this the second largest biopharmaceutical product category after vaccines. As the development of potential new therapeutic agents into commercial products takes about 10 years, the FDA-approved antibodies, and some of those in the end-stages of development pipelines, are chimeric or humanized antibodies that were developed with early antibody engineering technologies. The more recently developed reagents, on the other hand, are completely human antibodies that are derived from phage antibody libraries and transgenic mice[17–20].

Antibodies against infectious agents

In the pre-antibiotic era, SERUM THERAPY was widely used as treatment for infectious diseases such as anthrax, smallpox, meningitis and the plague. With the introduction of vaccines and antibiotics, the use of serum therapy declined. However, with the reduction and eradication of certain diseases (for example, smallpox), which has had the result that populations are no longer routinely vaccinated against these infectious agents, and the increasing emergence of antibiotic-resistant bacteria, humans are susceptible to acute outbreaks of disease or to biological terrorism. Antibiotics are obviously ineffective at eliminating viral infections, and the antiviral drugs in use today are often associated with a short serum half-life and resistance often emerges after repeated use[21]. Passive antibody serum therapy is today used merely in replacement therapy for patients with immune disorders, for POST-EXPOSURE PROPHYLAXIS against several viruses (for example, rabies, measles, hepatitis A and B, varicella and respiratory syncitial virus (RSV)) and for toxin neutralization (for example, diphtheria, botulism and tetanus). Passive immunization has substantial advantages over the administration of antimicrobial agents, including low toxicity and high specific

EFFECTOR FUNCTIONS
The antigen-elimination processes mediated by immunoglobulins and initiated by the binding of effector molecules to the Fc part of the immunoglobulin. The common effector functions are complement-dependent cytotoxicity (CDC), phagocytosis and antibody-dependent cellular cytotoxicity (ADCC).

COMPLEMENT-DEPENDENT CYTOTOXICITY
Once bound to antigen, both IgM and IgG can trigger a sequence of reactions by which serum proteins called complement factors are cleaved. One of the results is destruction of the target cell through complement-dependent cytotoxicity.

PROGRAMMED CELL DEATH
Programmed cell death infers that cells are determined to die at a specific stage of development or having received a specific signal. The process is known as apoptosis. The cells shrivel and are engulfed by nearby phagocytic cells without eliciting any inflammatory response.

© 2002 Nature Publishing Group

Samsung Bioepis Exhibit No. 1002
Page 911

Box 1 | Immunoglobulin classes

Antibodies belong to either one of five immunoglobulin (Ig) classes: IgA, IgD, IgE, IgG or IgM. Each class has a distinct structure and biological activity. Some of the classes are further divided into subclasses — for example, there are four IgG subclasses and two IgA subclasses. IgM is the first antibody to be produced in an immune response and forms a pentameric complex comprised of Ig monomers. IgA is the main class of antibody in external secretions, where it is found as a dimer that protects the body's mucosal surfaces from infection; it is also found as a monomer in serum. IgD is the main antibody on the surface of B cells. IgE is found bound to cells that secrete histamines after antigen binding. IgG is the main antibody in serum. The IgG class is the most stable and has a serum half-life of 20 days, whereas IgM and IgA persist for only 5–8 days. Both IgM and IgG can mediate complement fixation, whereas only IgG can promote antibody-dependent cellular cytotoxicity (ADCC). IgA, and to a certain degree IgM, can mediate trancytosis to mucosal surfaces, whereas only IgG can be transported across placenta for fetal protection.

activity, as well as an immediate effect compared with vaccines and even antibiotics. Given these issues, biotechnology companies and institutions working within the field of infectious disease protection are certain to direct efforts towards developing highly effective and functional antibody candidates against specific disease targets.

The isolation of protective toxin-neutralizing human monoclonal antibodies was described in 1993 in a study in which several human monoclonal antibodies against tetanus toxin were isolated and a protective effect against tetanus toxin was observed[22]. However, when combinations of these specific monoclonal antibodies were administered, an extra potent (that is, synergistic) effect was observed. Such an effect has also recently been described in the neutralization of botulinum neurotoxin. The anti-botulinum toxin antibodies were derived from different phage-display libraries obtained from humans or immunized mice (J. Marks, presented at Cambridge Healthtech Institute conference on Recombinant Antibodies, Cambridge, Massachusetts, USA, 24–25 April 2002). Recently, Maynard and colleagues described high-affinity antibodies against *Bacillus anthracis*[23]. These antibodies were fragments derived from variable-chain genes of a mouse monoclonal antibody and expressed in *E. coli* as single-chain Fv fragments (scFvs). By administering the antibody fragments to mice before injection of anthrax toxin, protection against the toxin was observed. Protective human antibodies against Shiga-toxin1 were recently isolated by the immunization of transgenic mice[20]. The panel of ten different antibodies of the IgM and IgG1 class that showed specificities to different subunits of the toxin effectively neutralized the toxin.

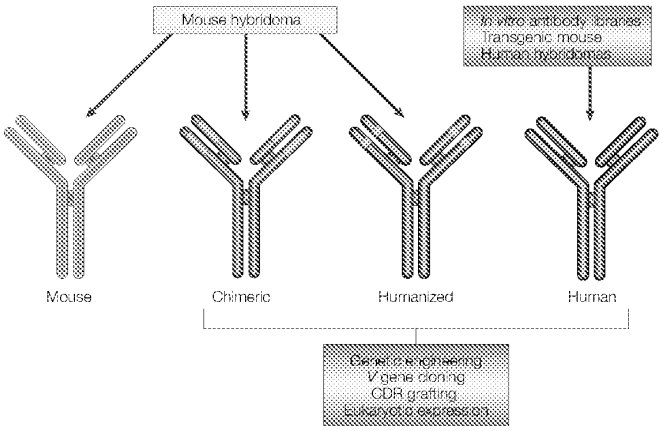


Figure 2 | **Antibody engineering.** Mouse hybridoma technology generates mouse monoclonal antibodies. Genetic engineering has fostered the generation of chimeric, humanized and human antibodies. Cloning of mouse variable genes into human constant-region genes generates chimeric antibodies. Humanized antibodies are generated by the insertion of mouse complementarity-determining regions (CDRs) onto human constant and variable domain frameworks; however, additional changes in the framework regions have, in several cases, been shown to be crucial in maintaining identical antigen specificity[75,76]. Fully human antibodies can be generated by the selection of human antibody fragments from *in vitro* libraries (see BOX 2 and FIG. 3a), by transgenic mice (FIG. 3b) and through selection from human hybridomas.

Compared with toxin neutralization, the use of antibodies in the prevention of viral disease is a more complex prospect. Palivizumab (Synagis; MedImmune Inc.) is a humanized IgG1 monoclonal antibody approved for the prevention of RSV infections in high-risk infants[17,24]. Palivizumab was the first monoclonal antibody approved for an infectious pathogen and is, so far, the only antiviral monoclonal antibody in clinical use. The development of sevirumab (Protovir; Protein Design Labs) — a human anti-cytomegalovirus (CMV) antibody — was, by contrast, halted in Phase III clinical trials as a supplemental treatment for CMV-induced retinitis because of a lack of evidence of efficacy. The elimination of a viral infection requires that a number of events occur, including inhibition of cell infection, mediation of cell killing of infected cells, inhibition of viral replication, inhibition of viral release and inhibition of cell–cell transmission[25,26]. Within the multitude of antibody specificities generated in the typical human polyclonal response, it is likely that one or several effective antibodies against one or more of the particular processes listed will be found. Some of these antibodies bind neutralizing epitopes, whereas others bind non-neutralizing epitopes. The overall outcome might indeed be effective protection due to the combination of the blocking, neutralizing and eliminating effect of human antibodies. Recently, XTL Pharmaceuticals reported on Phase I/II clinical trials with two human monoclonal antibodies against hepatitis B virus[28]. The two monoclonal antibodies were combined with lamivdudine (Epivir-HBV; GlaxoWellcome) — an antiviral drug that inhibits DNA replication — and showed significant reduction in serum viral titre. If monoclonal antibodies are to be used as prophylactics or therapeutics against infectious diseases, it is likely that their efficacy will be increased when a polyclonal passive human serum therapy is mimicked — that is, when a pool of highly specific and high-affinity monoclonal antibodies are administered. There are, however, limitations to this approach, including the production costs associated with manufacturing intact human antibodies.

© 2002 Nature Publishing Group

Samsung Bioepis Exhibit No. 1002
Page 912

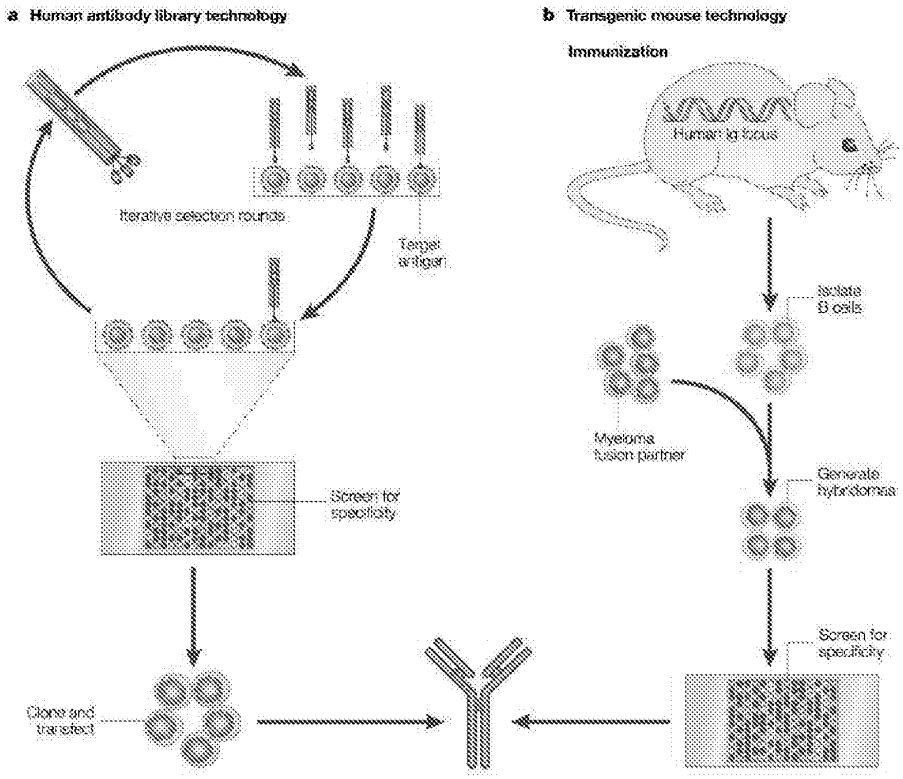


Figure 3 | ***In vitro* and *in vivo* human antibody techniques exemplified by phage display and transgenic mouse technologies. a** | The *in vitro* process is based on panning the library of antibodies against an immobilized target. The non-binding phage antibodies are washed away and the recovered antibodies are amplified by infection in *Escherichia coli*. The selection rounds are subsequently repeated until the desired specificity is obtained. The antibody format for screening is either Fab or single-chain Fv. The expression of antibodies in *E. coli* and recent developments in screening technologies[77] have made it possible to screen tens of thousands of clones for specificity. The antibody fragments themselves can be used as therapeutic agents as discussed in this review, but they can also be converted into intact immunoglobulins by the cloning of the variable genes into plasmids incorporating the constant-region genes of immunoglobulins. The genes are transfected into cell lines and therefore produce fully human immunoglobulins. **b** | The *in vivo* process is based on the immunization of a transgenic mouse. The mouse has been genetically engineered and bred for the expression of human immunoglobulins. The B cells harvested after immunization can be immortalized by fusion with a myeloma cell line, as in traditional hybridoma technology. The hybridomas can then be screened for specific antibodies.

HYBRIDOMA
An antibody-secreting B-cell line that is generated by fusing splenic-derived B cells with a plasmacytoma. A hybridoma produces the same antibody as the parent B cell and divides and grows in culture like the parent cancer cell. The antibody produced is monoclonal.

SERUM THERAPY
The treatment of an infectious disease with the serum from an immunized animal or individual, and which contains antibody.

POST-EXPOSURE PROPHYLAXIS
A treatment that is designed to protect an individual against a disease agent to which the individual has been recently exposed.

A possible solution to this problem is to instead use blocking antibody fragments synthesized in *E. coli*, which could be used to combat, for example, viral infections. A further difficulty in developing such multi-antibody therapeutics might arise from regulatory concerns regarding the administration of multiple monoclonal antibodies. The experience obtained from XTL Pharmaceuticals' use of combined antibodies might be an important step in resolving — or exacerbating — these concerns.

Anti-inflammatory antibodies

Antibodies with high specificity and affinity can be developed to bind specific cytokines or their receptors. In both cases, the purpose is to inhibit the detrimental effect of the cytokine. Cytokines associated with inflammation and autoimmunity include tumour-necrosis factor-α (TNF-α), interleukins and complement proteins. Modulation of immune-responses, such as immune-cell depletion by the targeting of antibodies to cell-surface receptors, for example, CD20 and CD4 on B or T cells, has also been shown as a viable therapeutic strategy in autoimmune diseases. Together with cancer, inflammatory and autoimmune diseases are an important focus for companies developing antibody therapies. In patients with rheumatoid arthritis (RA), TNF-α accumulates in the joints and contributes to the inflammation and joint destruction that is associated with the disease. Marketed products directed towards the regulation of TNF-α include the soluble TNF-α receptor etanercept (Enbrel; Amgen Inc/Wyeth) and the antibody infliximab

© 2002 Nature Publishing Group

Samsung Bioepis Exhibit No. 1002
Page 913

Table 1 | **Approved monoclonal antibodies**

Product	Year approved	Type of molecule	Disease indication	Company
OKT-3	1986	Murine (anti-CD3)	Organ transplant rejection	Ortho Biotech Products LP
ReoPro	1994	Chimeric (anti-platelet gpIIb/IIIa)	Coronary intervention and angioplasty	Centocor Inc
Panorex (Germany only)	1995	Murine (anti-EpCAM)	Colorectal cancer	Centocor Inc
Rituxan	1997	Chimeric (anti-CD20)	Non-Hodgkin's lymphoma	IDEC Pharmaceuticals Corp
Zenapax	1997	Humanized (anti-IL-2 receptor)	Refractory unstable angina	Centocor Inc
Herceptin	1998	Humanized (anti-ERBB2)	Metastatic breast cancer	Genentech Inc
Remicade	1998	Chimeric (anti-TNF-α)	Crohn's disease	Centocor Inc
Simulect	1998	Chimeric (anti-IL-2 receptor)	Kidney transplant rejection	Novartis AG
Synagis	1998	Humanized (anti-F-protein)	Respiratory syncitial viral disease	MedImmune Inc
Mylotarg	2000	Humanized (anti-CD33)	Chemotherapy for acute myeloid leukemia	Celltech Group plc/Wyeth
Campath	2001	Humanized (anti-CD52)	B-cell chronic lymphocytic leukemia	Millennium Pharmaceuticals/ Ilex Oncology Inc.
Zevalin	2002	Mouse (anti-CD20)	B-cell non-Hodgkin's lymphoma	IDEC Pharmaceuticals Corp
Xolair (Australia only)	2002	Humanized (anti-IgE Fc)	Allergy	Tanox Inc/Genentech Inc/ Novartis AG

Adapted and updated from REF. 81.

(Remicade; Centocor Inc). Infliximab is a chimeric antibody with mouse variable domains and human constant domains of the IgG1 subclass. There are a number of anti-TNF-α antibodies in clinical trials, including CDP571 and CDP870 (Celltech Plc) and adalimumab (D2E7; Abbot Laboratories/Cambridge Antibody Technology). Adalimumab is the first phage-display-derived human antibody brought into the clinic, and was generated by 'guided selection' using a mouse monoclonal antibody[28]. The method is based on the selection of a human variable-domain repertoire coupled to one of the original mouse variable domains, so as to 'guide' the human variable-domain repertoire towards the same specificity as the original mouse variable domains. The antibody is affinity optimized by iterative rounds of selection and mutagenesis (FIG. 3; BOX 2, 3). Adalimumab has completed Phase III clinical trials and is currently in registration for FDA approval. Recently, the anti-CD20 antibody rituximab (Rituxan; IDEC Pharmaceuticals; see below) completed Phase II clinical trials in RA and showed promising results compared with the anti-TNF-α antibodies adalimumab and infliximab[29], indicating that depletion of B cells might be an effective treatment in RA and other autoimmune diseases[30]. Eculizumab (5G1.1; Alexion Pharmaceuticals) is a humanized monoclonal antibody that prevents the cleavage of human complement component C5 into its pro-inflammatory components[31], whereas J695 (Cambridge Antibody Technology) is a human antibody derived from a phage-display library against interleukin-2 (see online links box). Both antibodies are in clinical trials at present and have shown potential in the treatment of inflammatory diseases such as RA and nephritis.

Antibodies targeting cancers

In cancer therapy, the purpose of antibody administration is to induce the direct or indirect destruction of cancer cells, either by specifically targeting the tumour or the vasculature that nourishes the tumour. Indeed, new technologies for the panning of antibody libraries on intact cells have made it possible to isolate antibodies against novel and promising cancer-associated antigens[32]. However, the most common cancer targets are the carcinoembryonic antigen (CEA), which is associated with colorectal cancers, MUC1, epidermal growth factor receptor (EGFR) and ERBB2 (also known as HER2/neu, associated with lung and breast cancer) and CD20 on B cells, which is a marker for non-Hodgkin's lymphoma (NHL). Examples of approved therapeutic antibodies directed towards ERBB2 and CD20 include the humanized IgG1 antibody trastuzumab (Herceptin; Genentech) for breast cancer and the chimeric IgG1 antibody rituximab (Rituxan; IDEC Pharmaceuticals) for NHL.

Today, specific antibody therapy is used in combination with classical chemotherapy, but the remaining challenge is to develop specific and highly cytotoxic drugs against cancer cells. Recently, the FDA approved two new antibody chemotherapies: ibritumomab tiuxetan (Zevalin; IDEC Pharmaceuticals) and gemtuzumab ozogamicin (Mylotarg; Wyeth). Ibritumomab tiuxetan is a mouse anti-CD20 antibody

© 2002 Nature Publishing Group

Samsung Bioepis Exhibit No. 1002
Page 914

attached to the radioisotope ^{90}yttrium that targets the surface of the mature B cells and B-cell tumours, inducing cellular damage in the target and neighbouring cells. Ibritumomab tiuxetan is the first radioimmunotherapeutic antibody approved by the FDA and is approved for use in the treatment of NHL. The antibody reacts with the same antigen as rituximab, but is a mouse antibody, which results in beneficial rapid clearance to decrease the undesired effect of total body irradiation. Gemtuzumab ozogamicin is a humanized monoclonal antibody that is linked to the antitumour agent calicheamicin, a bacterial toxin[33]. The antibody is targeted to CD33, which is expressed in about 90% of all acute myelogenic leukaemia (AML) cases, and has been approved for administration to patients who have relapsed AML. Both ibritumomab tiuxetan and gemtuzumab ozogamicin are examples of antibodies designed to specifically deliver their toxic load directly to cancer cells. Antibodies directed against major histocompatibility complex (MHC) class II proteins specifically target and eliminate cancer cells. The mouse monoclonal antibody Oncolym (Lym-1; Peregrine Pharmaceuticals)[34] and the antibody Hu1D10 (Remitogen; Protein Design Labs)[35] are two examples of such antibodies. Whereas one form of Oncolym is radiolabelled with ^{131}iodine (REF. 36), the humanized IgG1 Hu1D10 eliminates cancer cells by virtue of its natural effector functions (FIG. 4 and see below), and also induces apoptosis. Recently, an anti-MHC class II human antibody derived from an antibody phage-display library was shown to induce apoptosis of activated lymphoid cells[19]. Vascular endothelial growth factor (VEGF) is a potent cytokine for angiogenesis (the formation of blood vessels). Inhibitory agents, including specific antibodies, have been developed to block VEGF-stimulated angiogenesis as a strategy to inhibit tumour growth. One example of such an antibody is the humanized anti-VEGF antibody bevacizumab (Avastin; Genentech), which is in Phase III clinical trials for the treatment of breast cancer and colorectal cancer. Monoclonal antibodies are being continuously developed for cancer therapy, and most new antibody formats have demonstrated utility within this field.

Naked antibodies

Both trastuzumab and rituximab are 'naked antibodies', meaning that they do not have a radioisotope or toxin attached to them. The elimination of the target of these antibodies depends entirely on the recruitment of the body's own effector mechanisms, namely complement

Table 2 | **A selection of antibodies in clinical development**

Product	Stage (2002)	Type of molecule	Disease indication	Company
ABX-EGF	Phase II	Human (anti-EGF-R)	Non-small-cell lung cancer	Abgenix Inc/Immunex Corp
ABX-IL8	Phase II	Human (anti-IL-8)	Pulmonary disease, chronic obstructive bronchitis	Abgenix Inc/Immunex Corp
Eculizumab (5G1.1)	Phase IIb	Humanized (anti-C5)	Rheumatoid arthritis, lupus nephritis	Alexion Pharmaceutical Inc
D2E7	Phase III	Human (anti-TNF-α)	Rheumatoid arthritis	Cambridge Antibody Technology/ Abbot Laboratories
CAT-152	Phase II/III	Human (anti-TGF-β2)	Scarring following glaucoma surgery	Cambridge Antibody Technology
J695	Phase II	Human (anti-IL-2)	Autoimmune diseases including rheumatoid arthritis	Cambridge Antibody Technology/Abbot/ Wyeth Genetics Institute
Antegren (natalizumab)	Phase III	Humanized (anti-α-4 integrin)	Crohn's disease, multiple sclerosis, inflammatory bowel disease	Elan Pharmaceuticals Corp/Biogen Inc
Avastin (rhuMAb VEGF)	Phase III (anti-VEGF)	Humanized	Metastatic breast cancer, non-small-cell lung cancer	Genentech Inc
IDEC-151 (Clenoliximab)	Phase II	Primatized (anti-CD4)	Rheumatoid arthritis	IDEC Pharmaceuticals Corp
MEDI-507 (Siplizumab)	Phase II	Humanized (anti-CD2 Receptor)	Suppresses NK and T-cell function	MedImmune Inc
XTL-001 (pairs of monoclonals)	Phase I/II	Human (anti-HBV)	Hepatitis B virus neutralization	XTL Biopharmaceuticals Ltd
CDP870	Phase II	Fab fragment (anti-TNF-α)	Rheumatoid arthritis	Celltech Group plc
CDP571	Phase III	Humanized (anti-TNF-α)	Crohn's disease	Celltech Group plc
Hu1D10 (Remitogen)	Phase II	Humanized (anti-MHC class II)	Non-Hodgkin's Lymphoma	Protein Design Labs Inc

Adapted from REFS 34, 81 and selected company web sites.

© 2002 Nature Publishing Group

Samsung Bioepis Exhibit No. 1002
Page 915

Box 2 | ***in vitro* human antibody libraries**

The strategy of selecting antibodies from large repertoires is dependent on the coupling between genotype and phenotype — that is, the displayed protein (the antibody) carries its own encoding gene. As a result, the process enables the easy recovery of the DNA encoding the selected protein. The power of such antibody libraries lies in the fact that selection takes place *in vitro*, thereby making them excellent systems for isolating antibodies to certain drugs, potent toxins and haptens that would normally be impossible to raise within *in vivo* systems such as the mouse hybridoma technology.

In phage display, the variable genes encoding the antibody variable domains are fused to genes encoding bacteriophage coat proteins. The most commonly used phage protein is the pIII protein located at the tip of the long, thin filamentous phage M13. The system is highly effective and is used to isolate single-chain Fv or Fab fragments with specificity to almost any kind of antigen. The plasmid encoding the variable genes is packaged within the viral capsid and the expressed antibody is presented on the bacteriophage surface. Phage libraries are made from the human naive repertoire (that is, the IgM and IgD class) from pre-immunized donors or totally synthetic variable genes. As the repertoire is created *in vitro*, the technology also generates high-affinity human antibodies against human proteins. The process is based on panning the library against an immobilized target in a test tube (FIG. 3a). The non-binding phage antibodies are washed away and the recovered antibodies are amplified by infection in *E. coli*. The selection rounds are subsequently repeated until the desired specificity is obtained.

In messenger RNA (mRNA)-display technologies, such as the ribosomal display technology, antibody domains are linked to ribosomes attached to the mRNA that they are translating. This process takes place entirely *in vitro* by transcripton of a DNA library followed by *in vitro* translation of the mRNA library. The subsequently recovered mRNA transcripts are reverse transcribed and amplified by polymerase chain reaction (PCR) for the subsequent round of selection. The method has been successfully used for the affinity maturation and stability engineering of antibody fragments[58,70]. A similar technology called ProFusion is based on the covalent linkage of the synthesized proteins to the mRNA template via a puromycin linker[71]. Another potential display method applicable for antibody display is the Covalent Display Technology. In this method, the protein is fused to P2A, a bacteriophage DNA-nicking protein that covalently binds its own DNA[72].

activation and Fc-receptor-dependent responses (FIG. 4). Such effector functions can be abolished or augmented by mutagenesis of the Fc part of the antibody. Both trastuzumab and rituximab seem to exert their effects primarily through an Fc-receptor-dependent mechanism[37], and both are of the IgG1 subclass of antibodies, and thus bind all known Fcγ receptors (FcγRs). These receptors are located in the membrane of various effector cells, such as natural-killer (NK) cells, neutrophils, monocyte/macrophages, dendritic cells and B cells. In general, IgG-FcγR crosslinking leads to antibody-cell cytotoxicity or phagocytosis of the target cells by the effector cell (FIG. 4). In addition, depending on the nature of the target, the crosslinking caused by FcR interaction can itself induce signalling that leads to apoptosis. Although most of the FcγRs exert the functions mentioned above, one class of FcγRs, FcγRIIb, has a general inhibitory effect. Recently, a thorough investigation of the interaction between IgG and FcγRs led to the discovery of IgG1 variants with improved or decreased binding affinity for the FcγRs, including some with increased binding affinity for the activating receptors and decreased binding to the inhibiting ones[38]. So, variants can now be selected with desired combinations of functions.

Another approach to enhance the effector functions of antibodies is to engineer bispecific antibodies, which comprise two specificities, one for the cell to be eliminated and one for receptors on effector cells such as cytotoxic T cells (BOX 4). Strategies for the production of bispecific antibodies have been developed[39], and an important new approach in this field is the activation of polymorphonuclear leucocytes (PMNLs), the most numerous cytotoxic effector-cell population. These cells have Fc receptors for the antibody class IgA (Fcγ receptor, CD89) and this has elicited a growing interest in IgA[40], and bispecific antibodies that recruit PMNLs through CD89 binding have shown promise in *in vitro* studies[41].

A possible side effect of antibody therapy is the cytokine-release syndrome that is probably mediated through recruitment of the immune effector cells.

Box 3 | **Affinity maturation of antibodies**

In the mammalian immune system, antibodies undergo affinity maturation and through the development of memory B cells retain an antibody repertoire for subsequent encounter of an identical pathogen. Similarly, *in vitro* antibody technologies have enabled the selective alteration of the affinity of antibodies. Several approaches have been developed, but a common element to all of these is that they are developed under stringent selection regimes, including reducing the concentration of antigen, altering washing conditions (see BOX 2), changing temperature or eluting with free antigen. Methods of antibody affinity maturation are based on the principle of changing parts of, or the whole, antibody variable domains while simultaneously keeping the specificity. Most of the contribution of specific antibody binding resides within the variable heavy-chain domain and especially within the CDR 3 loops of both variable heavy and variable light chains (see FIG. 1).

Chain-shuffling is based on the method of substituting the native light chain with a new light-chain repertoire, but retaining the variable heavy chain. Panning of such chain-shuffled libraries in stringent conditions generates antibodies with higher affinities than the mother antibody clone. The randomization of CDR regions has also been used as a maturation strategy. Another approach is to generate an antibody library with mutations within the variable genes either by error-prone polymerase chain reaction, *E. coli* mutator strains or site-specific mutagenesis. Both phage display and ribosomal display have been successfully used for affinity maturation.

© 2002 Nature Publishing Group

Samsung Bioepis Exhibit No. 1002
Page 916

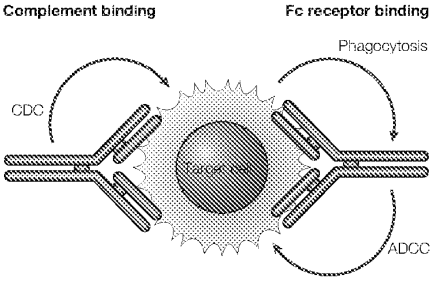


Figure 4 | **Natural immunoglobulin effector functions.** The classical complement pathway is initiated by the binding of C1 to immune complexes (that is, antibody-coated cells or antigens) and eventually leads to cellular lysis and elicits inflammatory responses for the ultimate elimination of the target cell. This process is called complement-dependent cytotoxicity (CDC). The Fc-receptor-mediated effector functions are associated with the recruitment of cellular effectors leading to either phagocytosis or antibody-dependent cellular cytotoxicity (ADCC). These mechanisms are accepted to be the processes that operate when naked antibodies are used as therapeutic agents.

In such cases, abrogation of Fc-dependent effector functions is desired and this can be achieved by mutating single amino acids in the Fc region of the antibodies[42].

Antibodies without Fc

Small antibody-derived fragments can do part of the job normally performed by intact antibodies, such as blocking the action of a toxin — for example, anthrax toxin[23] — or blocking the interaction between a cytokine and its receptor[43,44]. These antibody fragments can also carry effector molecules to their targets. The blocking effect of antibodies simply requires the inhibition of subsequent ligand or receptor binding, and does not necessarily need effector functions. However, even fragments with effector functions normally associated with 'naked antibodies' — the bispecific antibody fragments — have been described. These fragments comprise either the Fv or Fab portion of the antibody (FIG. 1), which in both cases is the part that binds antigen. Such antibody fragments can be functionally expressed in *E. coli* with ease, which is a prerequisite for antibody phage display as well as for other *in vitro* human antibody library approaches. One of the great advantages of the library approaches in general is that they allow for the selection of antibodies of high specificity and affinity towards a variety of different antigens (BOX 2). Engineered antibodies have therefore become smaller, with increasing affinity and specificity. Another obvious advantage is the ability to select specific antibodies against toxins, drugs, cytokines and other targets that cannot, for various reasons, be injected into an animal to raise an immune response, one of which is that the target might kill the animal or that it might not be immunogenic at all (BOX 2).

Coupling the antibody fragments into dimers and trimers generates oligovalent antibody fragments with increased avidity and the ability to crosslink target molecules (FIG. 5). In this approach, the antibody is first reduced to the small antigen-binding portion and then engineered to form a larger molecule to gain avidity and crosslinking ability. So, the signalling effect that requires crosslinking of receptors might also be achieved with antibody fragments. The so-called triabodies are antibody fragments in which the valency is increased to three antigen-binding sites[45,46], thereby increasing the avidity of the antibody fragments. Such multivalent antibody fragments might prove to be effective in the blocking and neutralization of toxins, as well as in the generation of targeting devices for cancer therapy.

Antibody fragments with effector functions normally associated with 'naked antibodies' — the diabodies — were first described in 1993 (REF. 47). Diabodies are scFv fragments with two different antigen specificities, one directed against the target and one directed against effector molecules such as complement[48] or Fc receptors[49]. Since then, diabodies have been designed in various ways to crosslink effector cells such as cytotoxic T cells[50,51] and NK cells[49], which is particularly useful for the treatment of lymphomas. Tandem diabodies[52], in which two diabodies are genetically coupled to achieve a better crosslinking effect, are still at an early stage of development. Another early-stage antibody format is pepbodies[53], which comprise Fab or scFv fragments linked to small peptides that bind effector molecules and thus mimic the way complete antibodies bind their effector molecules.

Antibody pharmacokinetics

A prerequisite for effective antibody targeting is that the antibodies should be able to penetrate tissues, and small antibody fragments are better than complete antibodies in this respect. Recent research has shown that high-affinity fragments are retained in the periphery of the tumour, whereas the medium-affinity antibodies penetrate throughout the tumour[54]. Furthermore, bivalent low-affinity fragments penetrate better and more uniformly than high-affinity fragments[55]. Fortunately, antibody fragments can be selected for optimal affinity and specificity by all of the *in vitro* selection processes[56,57]. The therapeutic efficacy of an antibody, however, also depends on its stability as well as its immunogenicity. Strategies for the engineering of antibody stability with regards to antibody fragments have been described[58].

Box 4 | **Strategies for making bispecific antibodies**

Bispecific antibodies have the ability to bind two different antigens and have been used to crosslink various cells and molecules, although they have been primarily used to re-direct effector cells to target cells (FIG. 5). Methods for generating bispecific antibodies tend to bias the production towards heterodimers — that is, towards two specificities instead of one. The first method was described in 1983 (REF. 73) in the generation of so-called hybrid-hybridomas, or quadromas, and is based on the fusion of two hybridoma cell lines, but this is a complex and time-consuming effort; chemical or genetic coupling of Fab fragments[74] is more effective. The inclusion of a C_H3 domain into antibody fragments has been used to function as a dimerization domain, and a modified form of a C_H3 domain has been used to direct hetero-dimerization[59]. Perhaps the most effective way to generate bispecific antibody fragments is by expression of bispecific single-chain Fv fragment as diabodies (FIG. 5).

© 2002 Nature Publishing Group

Samsung Bioepis Exhibit No. 1002
Page 917

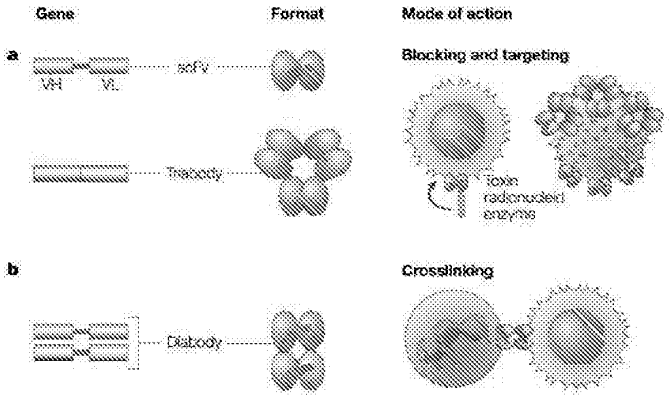


Figure 5 | **Different formats of antibody fragment. a** | Single-chain Fv fragments (scFvs) are mostly monomeric when the peptide linker (in red) between the V_H and the V_L domains is about 12–15 amino acids. When the linker is shorter than three amino acids or is totally deleted, the fragments tend to make trimeric fragments or even tetrameric formats. Multivalent antibodies gain avidity and such molecules are therefore perfect for blocking viral infection or receptor–ligand interactions, for example. **b** | If the linker is about five amino acids long, then the variable domains tend to dimerize. When equipped with variable domains with different specificities, such dimeric scFvs can make bispecific fragments called diabodies.

Similarly, the presumed immunogenicity of human antibodies can be reduced by mutagenesis of typical B-cell[59] or T-cell epitopes[60]. As the development of monoclonal antibodies moved away from mouse antibodies to chimeric antibodies and subsequently to humanized antibodies, a reduction in the level of anti-antibody responses was observed with new antibody products. It will, however, be interesting to observe the immunogenicity of fully human antibodies in therapeutic settings. In particular, a comparison of D2E7 with infliximab (that is, a human versus a chimeric anti-TNF-α monoclonal antibody) will provide valuable information in this respect. Small antibody fragments show rapid clearance from the circulation, and as a result the fraction of the injected dose that reaches its target is at present too low for a therapeutic benefit, even for bivalent fragments. Therefore, the manipulation of antibody fragments to increase their half-life will be crucial for the success of therapeutic antibody fragments.

Complete human antibodies have long serum half-lives owing to their ability to bind to the so-called neonatal Fc receptor (FcRn). This receptor both transports IgG across the placenta from mother to fetus and protects serum IgG from degradation. FcRn is expressed on placenta and blood-vessel linings and binds IgG in the junction between the C_H2 and C_H3 domains of the Fc region[61] (see FIG. 1). As Fab and scFv fragments lack the Fc region altogether, they are not rescued from degradation by this mechanism and, as a result, have a short serum half-life.

A new approach to increasing the half-life of antibodies is the PEGYLATION of antibodies and antibody fragments, which simultaneously reduces their immunogenicity[62]. The effect is achieved by chemical coupling of polyethylene glycol (PEG) to amino groups in the protein structure. The main property contributing to this effect is the increase in the size of the molecule above the glomerular-filtration limit. However, modifications of amino groups within antibodies affect the effector functions[63] as well as the antigen-binding capacity[64]. A possible alternative is to direct the PEG attachment to sites on the antibody molecules that are distant from the CDR regions and crucial sites for effector functions[62]. Indeed, an anti-TNF-α human Fab fragment (CDP 870; Celltech) has its circulating half-life prolonged to 14 days by site-specific pegylation in the hinge region[44].

PEGYLATION
The covalent attachment of the polymeric molecule polyethylene glycol (PEG) to proteins.

TROYBODY
An antibody with specificity for an antigen-presenting cell and with an antigenic fragment inserted into a constant domain.

The potentiation of immune responses

Antibodies are the perfect high-specificity targeting and delivery devices. However, in the immune responses mounted by the body in response to pathogenic organisms and cancer, the cellular response mediated by T cells is a crucial component. Infectious diseases are eliminated by the combined action of antibodies and T cells. In the search for optimized vaccines, the aim is to use agents that both stimulate antibody and T-cell responses. A discussion of the immune effects of T cells is beyond the scope of this review, although new antibody-based technologies that aim at modulating T-cell responses are important issues for the potentiation of a desired therapeutic effect by, for example, therapeutic antibodies. One strategy for increasing the immune response is to load antibodies with antigenic material in the CDR regions[65]. This strategy is one of several that aim to use the Fc portion of the antibody to load specialized immune cells with antigenic material. In the TROYBODY strategy[66], the antibodies carry pieces of antigenic material as part of their Fc region and target the specialized immune cells by way of their Fab regions. In all cases, the purpose of the immune-cell loading is to activate disease-specific helper T cells. Indeed, Troybodies increase such T-cell responses more than 10,000-fold compared with the administration of the antigenic material in free form[67,68]. This strategy requires that the antigenic material is characterized, which is a significant challenge. An advantage of this approach, however, is that it can be combined with strategies that aim at activating the cytotoxic T cells that are crucial in the destruction of cancer and virus-infected cells. Recently, myeloma cells were coated with antibodies specific for syndecan-1 (SDC1), an antigen that is highly expressed on these tumour cells, which promoted the uptake of the tumour cells by dendritic cells through an Fc-receptor-dependent mechanism and the subsequent stimulation of tumour-specific cytotoxic T cells[69].

Concluding remarks

Throughout the development of therapeutic antibodies over the past 30 years, many obstacles have been overcome and, today, therapeutic antibodies have entered a new era. It is no longer a significant effort to select human antibodies against any kind of disease target. The first antibody derived from phage antibody

© 2002 Nature Publishing Group

Samsung Bioepis Exhibit No. 1002
Page 918

libraries has recently completed Phase III trials, and there are, at the moment, several other antibodies derived from both *in vitro* libraries and transgenic mice in clinical trials. There is a rising awareness of antibody effector functions and efforts are being directed towards modulating Fc-associated functions, such as complement activation and Fc-receptor activity. A remaining impediment to the development of new antibody therapeutics by the pharmaceutical industry is the cost of producing antibodies with mammalian expression systems. The new small therapeutic antibody formats, such as scFv, Fab or diabodies, can, however, be produced in *E. coli* at a much lower cost, and even antibody production in plants is a growing field. As antibodies are such versatile and highly specific molecules, fine tuned by millions of years of evolution to be optimal agents for targeted disease elimination, their development for various indications will, no doubt, be pursued with vigour for the foreseeable future.

1. Köhler, G. & Milstein, C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **256**, 495–497 (1975).
2. Larrick, J. W. & Fry, K. E. Recombinant antibodies. *Hum. Antibodies Hybridomas* **2**, 172–189 (1991).
3. Khazaeli, M. B., Conry, R. M. & LoBuglio, A. F. Human response to monoclonal antibodies. *J. Immunother.* **15**, 42–52 (1994).
4. Morrison, S. L., Johnson, M. J., Herzenberg, L. A. & Oi, V. T. Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. *Proc. Natl Acad. Sci. USA* **81**, 6851–6855 (1984).
5. Boulianne, G. L., Hozumi, N. & Shulman, M. J. Production of functional chimaeric mouse/human antibody. *Nature* **312**, 643–646 (1984).
6. Bell, S. & Kamm, M. The clinical role of anti-TNFα antibody treatment in Crohn's disease. *Aliment. Pharmacol. Ther.* **14**, 501–514 (2000).
7. Jones, P. T., Dear, P. H., Foote, J., Neuberger, M. S. & Winter, G. Replacing the complementarity-determining regions in a human antibody with those from a mouse. *Nature* **321**, 522–525 (1986).
8. Skerra, A. & Plückthun, A. Assembly of a functional immunoglobulin F_v fragment in *Escherichia coli*. *Science* **240**, 1038–1041 (1988).
9. Better, M., Chang, C. P., Robinson, R. R. & Horwitz, A. H. *Escherichia coli* secretion of an active chimeric antibody fragment. *Science* **240**, 1041–1043 (1988).
10. McCafferty, J., Griffiths, A. D., Winter, G. & Chiswell, D. J. Phage antibodies: filamentous phage displaying antibody variable domains. *Nature* **348**, 552–554 (1990).
11. Green, L. L. Antibody engineering via genetic engineering of the mouse: XenoMouse strains are a vehicle for the facile generation of therapeutic human monoclonal antibodies. *J. Immunol. Methods* **231**, 11–23 (1999).
12. Craig, M. S., French, R. R. & Glennie, M. J. Signalling antibodies in cancer therapy. *Curr. Opin. Immunol.* **11**, 541–547 (1999).
13. Farah, R. A., Cinchy, B., Herrera, L. & Vitetta, E. S. The development of monoclonal antibodies for the therapy of cancer. *Crit. Rev. Eukaryotic Gene Expr.* **8**, 321–356 (1998).
14. Berard, J. L. A review of interleukin-2 receptor antagonists in solid organ transplantations. *Pharmacotherapy* **19**, 1127–1137 (1999).
15. Maini, R. *et al.* Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. *Lancet* **254**, 1932–1939 (1999).
16. Sandborn, W. J. & Hanauer, S. B. Antitumor necrosis factor therapy for inflammatory bowel disease: a review of agents, pharmacology, clinical results and safety. *Inflamm. Bowel Dis.* **5**, 119–133 (1999).
17. Saez-Llorens, X. *et al.* Safety and pharmacokinetics of an intramuscular humanised antibody monoclonal antibody to respiratory syncitial virus in premature infants with bronchopulmonary dysplasia. *Pediatr. Infect. Dis.* **17**, 787–791 (1998).
18. Huls, G. A. *et al.* A recombinant, fully human monoclonal antibody with antitumor activity constructed from phage-displayed antibody fragments. *Nature Biotechnol.* **17**, 276–280 (1999).
19. Nagy, Z. A. *et al.* Fully human, HLA-DR-specific monoclonal antibodies efficiently induce programmed death of malignant lymphoid cells. *Nature Med.* **8**, 801–807 (2002).
20. Mukherjee, J. *et al.* Production and characterization of protective human antibodies against shiga toxin 1. *Infect. Immun.* **70**, 5886–5899 (2002).
21. Zeitlin, L., Cone, R. A., Moench, T. R. & Whaley, K. J. Preventing infectious disease with passive immunization. *Microbes Infect.* **2**, 701–708 (2000).
22. Lang, A. B., Cryz, S. J. Jr, Schurch, U., Ganss, M. T. & Bruderer, U. Immunotherapy with human monoclonal antibodies. Fragment A specificity of polyclonal and monoclonal antibodies is crucial for full protection against tetanus toxin. *J. Immunol.* **151**, 466–472 (1993).
23. Maynard, J. A. *et al.* Protection against anthrax toxin by recombinant antibody fragments correlates with antigen affinity. *Nature Biotechnol.* **20**, 597–601 (2002).
24. Groothuis, J. R., Nishida, H. Prevention of respiratory syncytial virus infections in high-risk infants by monoclonal antibody (palivizumab). *Pediatr. Int.* **44**, 235–241 (2002).
25. Parren, P. W. H. I. & Burton, D. R. The anti-viral activity of antibodies *in vitro* and *in vivo*. *Adv. Immunol.* **77**, 195–262 (2001).
26. Burton, D. R. Antibodies, viruses and vaccines. *Nature Rev. Immunol.* **2**, 706–713 (2002).
An excellent review on the use of antibodies against viral diseases and the use of donor antibodies as tools for the development of recombinant vaccines, a process termed 'reverse vaccinology'.
27. Eren, R. *et al.* Clinical evaluation (phase I) of a combination of two human monoclonal antibodies to HBV: safety and antiviral properties. *Hum. Antibodies* **11**, 27–28 (2002).
28. Jespers, L. S., Roberts, A., Mahler, S. M., Winter, G. & Hoogenboom, H. R. Guiding the selection of human antibodies from phage display repertoires to a single epitope of an antigen. *Biotechnology* **12**, 899–903 (1994).
29. Haan, K. Product development: Rituxan's challenge in RA. *Biocentury* **10**, A1–A5. (2002).
30. Looney, R. J. Treating human autoimmune disease by depleting B-cells. *Ann. Rheum. Dis.* **61**, 893–896 (2002).
31. Keating, M. Ecuilizumab (Alexion). *Curr. Opin. Investig. Drugs* **3**, 1017–1023 (2002).
32. Roovers, R. C., Van der Linden, E., De Bruine A. P., Arends, J. W. & Hoogenboom, H. R. Identification of colon tumour-associated antigens by phage antibody selection on primary colorectal carcinoma. *Eur. J. Cancer* **37**, 542–549 (2001).
33. Larson, R. A. *et al.* Antibody-targeted chemotherapy of older patients with acute myeloid leukemia in first relapse using Mylotarg (gemtuzumab ozogamicin). *Leukemia* **16**, 1627–1636 (2002).
34. Epstein, A. L. *et al.* Two new monoclonal antibodies, Lym-1 and Lym-2, reactive human with B-lymphocyte and derived tumors with immunodiagnostic and immunotherapeutic potential. *Cancer Res.* **47**, 127–135 (1987).
35. Ginaldi, R. D., Dahle, C. E., Hoskins, K. F. & Sennett, M. J. Identification and characterization of a new surface membrane found predominantly on malignant B-lymphocytes. *Blood* **75**, 2375–2387 (1990).
36. deNardo, S. J. *et al.* Treatment of B-cell malignancies with ^{131}I Lym-1 monoclonal antibodies. *Int. J. Cancer Suppl.* **3**, 96–101 (1988).
37. Clynes, R. A., Towers, T. L., Presta, L. G. and Ravetech, J. V. Inhibitory Fc receptors modulate *in vivo* cytoxicity against tumor targets. *Nature Med.* **6**, 443–446 (2000).
38. Shields, R. L. *et al.* High resolution mapping of the binding site on human IgG1 for FcγRI, FcγRII, FcγRIII, and FcRn and design of IgG1 variants with improved binding to the FcγR. *J. Biol. Chem.* **276**, 6591–6604 (2000).
39. Carter, P. Bispecific human IgG by design. *J. Immunol. Methods* **248**, 7–15 (2001).
40. Dechant, M. & Valerius, T. IgA antibodies for therapy. *Crit. Rev. Oncol. Hematol.* **39**, 69–77 (2001).
41. Repp, R. & Valerius, T. Bispecific antibodies targeting cancer cells. *Biochem. Soc. Transactions* **30**, 507–511 (2002).
42. Presta, L. G. Engineering antibodies for therapy. *Curr. Pharm. Biotechnol.* **3**, 237–256 (2002).
Whereas the focus for therapeutic antibodies has mostly been directed towards the antigen-binding part of antibodies, this paper describes the very important aspect of antibody effector functions and the modulation of the *in vivo* actions of antibodies.
43. Weir, A. N. C. *et al.* Formatting antibody fragments to mediate specific therapeutic functions. *Biochem. Soc. Transactions* **30**, 512–516 (2002).
44. Choy, E. H. *et al.* Efficacy of a novel PEGylated humanized anti-TNF fragment (CDP870) in patients with rheumatoid arthritis: a phase II double-blinded, randomized, dose-escalating trial. *Rheumatology* **41**, 1133–1137 (2002).
45. Iliades, P., Kortt, A. A. & Hudson, P. J. Triabodies: single chain Fv fragments without a linker form trivalent trimers. *FEBS Lett.* **409**, 437–441 (1997).
46. Kortt, A. A., Dolezal, O., Power, B. E. & Hudson, P. J. Dimeric and trimeric antibodies: high avidity scFvs for cancer targeting. *Biomol. Eng.* **18**, 95–108 (2001).
47. Holliger, P., Prospero, T. & Winter, G. "Diabodies": small bivalent and bispecific antibody fragments. *Proc. Natl Acad. Sci. USA* **90**, 6444–6448 (1993).
The pivotal paper describing diabodies. Since the publication of this paper, the concept has been used and developed by many groups and has become a means for generating bispecific antibodies.
48. Kontermann, R. E., Wing, M. G. & Winter, G. Complement recruitment using bispecific diabodies. *Nature Biotechnol.* **15**, 629–631 (1997).
49. Kipriyanov, S. M. *et al.* Synergistic antitumor effect of bispecific CD19 x CD3 and CD19 x CD16 diabodies in a preclinical model of non-Hodgkin's lymphoma. *J. Immunol.* **169**, 137–144 (2002).
50. Holliger, P., Prassinck, J., Williams, R. L., Thielemans, K. & Winter, G. Specific killing of lymphoma cells by cytotoxic T-cells mediated by a bispecific diabody. *Protein Eng.* **9**, 299–305 (1996).
51. Cochlovius, B. *et al.* Treatment of human B cell lymphoma xenografts with a CD8 x CD19 diabody and T cells. *J. Immunol.* **165**, 888–895 (2000).
52. Kipriyanov, S. M. *et al.* Bispecific Tandem diabody for tumor therapy with improved antigen binding and pharmacokinetics. *J. Mol. Biol.* **293**, 41–56 (1999).
53. Lunde, E. *et al.* Trybodies and Pepbodies. *Biochem. Soc. Trans.* **30**, 500–506 (2002).
54. Adams, G. P. *et al.* High affinity restricts the localization and tumor penetration of single-chain fv antibody molecules. *Cancer Res.* **61**, 4750–4755 (2001).
55. Nielsen, U. B., Adams, G. P., Weiner, L. M. & Marks, J. D. Targeting of bivalent anti-ErbB2 diabody antibody fragments to tumor cells is independent of the intrinsic antibody affinity. *Cancer Res.* **60**, 6434–6440 (2000).
56. Schier, R. *et al.* Isolation of high-affinity monomeric human anti-c-erbB-2 single chain Fv using affinity-driven selection. *J. Mol. Biol.* **255**, 28–43 (1996).
57. Hanes, J., Schaffitzel, C., Knappik, A. & Plückthun, A. Picomolar affinity antibodies from a fully synthetic naive library selected and evolved by ribosome display. *Nature Biotechnol.* **18**, 1287–1292 (2000).
Describes the selection of antibody fragments with the highest affinities reported so far. This also validates the ribosomal-display technology (see Box 2) as a potential tool for affinity maturation of antibodies.
58. Wörn, A. & Plückthun, A. Stability engineering of antibody single-chain Fv fragments. *J. Mol. Biol.* **305**, 989–1010 (2001).
A thorough review describing different aspects of protein stability of antibody fragments and the successful engineering applications of antibodies to increase their stability.
59. Padlan, E. A. A possible procedure for reducing the immunogenicity of antibody variable domains while preserving their ligand-binding properties. *Mol. Immunol.* **28**, 489–498 (1991).
60. Adair, F. Monoclonal antibodies: magic bullets or a shot in the dark. *Drug Disc. World* **3**, 53–59 (2002).

© 2002 Nature Publishing Group

Samsung Bioepis Exhibit No. 1002
Page 919

61. Ghetie, V. & Ward, E. S. Multiple roles for the major histocompatibility complex class I- related receptor FcRn. *Annu. Rev. Immunol.* **100**, 245–251 (2000).
62. Chapman, A. P. PEGylated antibodies and antibody fragments for improved therapy: a review. *Adv. Drug Deliv. Rev.* **54**, 531–545 (2002).
63. Anderson, W. L. & Tomasi T. B. Polymer modification of antibody to eliminate immune complex and Fc binding. *J. Immunol. Methods* **109**, 37–42 (1988).
64. Suzuki, T., Kanbara, N., Tomono, T., Hayashi N. & Shinohara, I. Physiochemical and biological properties of poly (ethylene glycol)-coupled immunoglobulin G. *Biochim. Biophys. Acta* **788**, 248–255 (1984).
65. Bona, C. A., Casares, S. & Brumeanu, T. D. Towards development of T cell vaccines. *Immunol. Today* **19**, 126–133 (1998).
66. Lunde, E. *et al.* "Troy-bodies": recombinant antibodies that target T cell epitopes to antigen presenting cells. *Int. Rev. Immunol.* **20**, 647–673 (2001).
67. Lunde, E., Munthe, L. A., Vabo, A., Sandlie, I. & Bogen, B. Antibodies engineered with IgD specificity efficiently deliver integrated T-cell epitopes for antigen presentation by B cells. *Nature Biotechnol.* **17**, 670–675 (1999).
68. Rasmussen, I. B., Lunde, E., Michaelsen, T. E., Bogen, B. & Sandlie, I. The principle of delivery of T cell epitopes to antigen-presenting cells applied to peptides from influenza virus, ovalbumin, and hen egg lysozyme: implications for peptide vaccination. *Proc. Natl Acad. Sci. USA.* **98**, 10296–10301 (2001).
69. Dhodapkar, K. M., Krasovsky, J., Williamson, B. & Dhodapkar, M. V. Antitumor monoclonal antibodies enhance cross-presentation of cellular antigens and the generation of myeloma-specific killer T cells by dendritic cells. *J. Exp. Med.* **195**, 125–133 (2002).
70. Irving, R. A., Coia G., Roberts, A., Nuttall, S. D. & Hudson, P. J. Ribosome display and affinity maturation: from antibodies to single V-domains and steps towards cancer therapeutics. *J. Immunol. Methods.* **248**, 31–45 (2001).
71. Xu, L. *et al.* Directed evolution of high-affinity antibody mimics using mRNA display. *Chem. Biol.* **9**, 933–942 (2002).
72. Fitzgerald, K. *In vitro* display technologies: new tools for drug discovery. *Drug Discov. Today* **5**, 253–258 (2000).
73. Milstein, C. & Cuello, A. C. Hybrid hybridomas and their use in immunohistochemistry. *Nature* **305**, 537–540 (1983).
74. Glennie, M. J., McBride, H. M., Worth A. T. & Stevenson, G. T. Preparation and performance of bispecific F(ab' γ)2 antibody containing thioether-linked Fab' γ fragments. *J. Immunol.* **139**, 2367–2375 (1987).
75. Kettleborough, C. A., Saldanha, J., Heath V.J. Morrison, C. J. & Bendig, M. M. Humanization of a mouse monoclonal antibody by CDR-grafting: the importance of framework residues on loop conformation. *Protein Eng.* **4**, 773–783 (1991).
76. Saldanha, J. W., Martin, A. C. & Leger, O. J. A single backmutation in the human kIV framework of a previously unsuccessfully humanized antibody restores the binding activity and increases the secretion in cos cells. *Mol. Immunol.* **36**, 709–719 (1999).
77. de Wildt, R. M., Mundy, C. R., Gorick, B. D. & Tomlinson, I. M. Antibody arrays for high-throughput screening of antibody-antigen interactions. *Nature Biotechnol.* **18**, 989–994 (2000). **Describes a method for screening thousands of antibody-producing colonies. Further refinement and the use of this method will certainly be important in the antibody discovery field.**
78. Das, R. C. & Morrow, K. J. Jr. *Antibody Therapeutics: Production, Clinical Trials and Strategic Issues.* (D&MD Publications, 2001).

Online links

DATABASES

The following terms in this article are linked online to:

Cancer.gov: http://www.cancer.gov/cancer_information/
acute myeloid leukemia | breast cancer | lung cancer | non-Hodgkin's lymphoma | non-small-cell lung cancer

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
Epidermal growth factor receptor | ERBB2 | MUC1 | SDC1 |

OMIM: http://www.ncbi.nlm.nih.gov/Omim/
Crohn's disease | rheumatoid arthritis

FURTHER INFORMATION

ClinicalTrials.gov: http://www.clinicaltrials. gov/

Cambridge Antibody Technology:
http://www.cambridgeantibody.co.uk

Access to this interactive links box is free online.

© 2002 Nature Publishing Group

All in-text references underlined in blue are linked to publications on ResearchGate, letting you access and read them immediately.

Samsung Bioepis Exhibit No. 1002
Page 920

Eculizumab found to be well tolerated and efficacious during 52 weeks of treatment in PNH

Publication info: ESPICOM Pharmaceutical and Medical Device News (Jan 2, 2007).
ProQuest document link

Full text: At the American Society of Hematology 48th Annual Meeting and Exposition, held in December 2006, results from the global, open-label Phase III SHEPHERD trial were presented. The data showed that Soliris (eculizumab; formerly known as 5G1.1), a novel humanised monoclonal antibody investigational drug developed by Alexion Pharmaceuticals, appeared to be safe and well tolerated, plus provided clinically and statistically significant improvements in intravascular haemolysis, anaemia, fatigue and quality of life in patients with paroxysmal nocturnal haemoglobinuria (PNH) during 52 weeks of treatment. According to investigators in the trial, the improvements made with eculizumab treatment occurred in a diverse and heterogeneous population of patients with PNH, which suggests the potential of the drug in the treatment of this disorder.
During the study, eculizumab was found to be well tolerated, with an adverse event (AE) profile similar to that of placebo-treated patients in the Phase III TRIUMPH study. Further, no serious AEs were deemed probably or definitely related to treatment. The most common AEs reported with eculizumab treatment in SHEPHERD were: headache, nasopharyngitis, nausea and upper respiratory tract infection. These events were generally consistent with those observed during six months of eculizumab and placebo treatment in TRIUMPH.
Eculizumab therapy reduced intravascular haemolysis, as shown by a reduction in the median lactate dehydrogenase area under the curve (-632,264 U/L W day; p<0.001). LDH levels during the study were reduced by 87 per cent, from a median of 2051 U/L at baseline to 269 U/L after 52 weeks of treatment (p<0.001). Clinically and statistically significant improvements in fatigue were observed as measured by change from baseline using the Functional Assessment of Chronic Illness Therapy-Fatigue instrument (p<0.001) and the fatigue scale of the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) instrument (p<0.001).
Treatment with eculizumab also significantly improved overall health and patient functioning as measured by EORTC QLQ-C30, including the global health status scale (p<0.001) and all five aspects of patient functioning: role (p<0.001), social (p<0.001), cognitive (p<0.001), physical (p<0.001) and emotional (p<0.001). Treatment also significantly improved seven of nine EORTC QLQ-C30 symptom scales and single-item measures, including pain (p<0.001), dyspnoea (p<0.001), appetite loss (p<0.001) and insomnia (p<0.001).
Clinically and statistically significant improvements in anaemia were also observed with eculizumab therapy, as evidenced by the reduction in transfusion requirements from a median of eight packed red cells in the 12-month pretreatment period to zero units during the 12 months of treatment (p<0.001). Additionally, 51 per cent of patients were transfusion independent for the entire 12-month treatment period (p<0.001). Other improvements in anaemia included a 44 per cent increase in the endogenous PNH red blood cell mass (p<0.001) and an increase in haemoglobin levels from 9.2g/dL at baseline to 10.2g/dL after 12 months of treatment (p<0.001).
Alexion also reported the initiation of an Expanded Access Program for eculizumab in the US for patients with PNH, in accordance with a Treatment Protocol, entitled EMBRACE, which was authorised by the FDA and expected to begin immediately. It is anticipated that treatment in EMBRACE will continue until marketing approval.
Eculizumab, a long-acting C5 complement inhibitor, is designed to selectively block terminal complement activation, thereby preventing the destruction of red blood cells by complement in patients with PNH. There is currently no therapy specifically available for the treatment of PNH.

Subject: Pharmaceutical

Language: English

Document type: Article

Publication title: ESPICOM Pharmaceutical and Medical Device News

Publication type: Newsletter

Publication date: Jan 2, 2007

Source attribution: ESPICOM Pharmaceutical and Medical Device News, © Publisher specific

Accession number: 00278402

Document URL: http://dialog.proquest.com/professional/docview/765088762?accountid=157282

First available: 2010-11-15

Database: ESPICOM Pharmaceutical &Medical Device News (2000 - current)

Contact ProQuest
Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

Inhibition of complement, neutrophil, and platelet activation by an anti-factor D monoclonal antibody in simulated cardiopulmonary bypass circuits

Michael Fung, PhD[a]
Paul G. Loubser, MD[b*]
Akif Ündar, PhD[c,d,e]
Maryann Mueller, CCP[d]
Cecily Sun, MS[a]
William N. Sun, PhD[a]
William K. Vaughn, PhD[e]
Charles D. Fraser, Jr, MD[c,d,e]

Objectives: Patients undergoing cardiopulmonary bypass frequently manifest generalized systemic inflammation and occasionally manifest serious multiorgan failure. Inflammatory responses of bypass are triggered by contact of blood with artificial surfaces of the bypass circuits, surgical trauma, and ischemia-reperfusion injury. We studied the effects of specific inhibition of the alternative complement cascade by using an anti-factor D monoclonal antibody (166-32) in extracorporeal circulation of human whole blood used as a simulated model of cardiopulmonary bypass.

Methods: Five healthy blood donors were used in the study. Monoclonal antibody 166-32 was added to freshly collected, heparinized human blood recirculated in a pediatric cardiopulmonary bypass circuit at a final concentration of 18 μg/mL. An irrelevant monoclonal antibody was used as a negative control with the same donor blood in a parallel bypass circuit on the same day. Blood samples were collected at different time points during recirculation for measurement of activation of complement, neutrophils, and platelets by immunofluorocytometric methods and enzyme-linked immunosorbent assays.

Results: Monoclonal antibody 166-32 inhibited the alternative complement activation and the production of Bb, C3a, sC5b-9, and C5a. Upregulation of CD11b on neutrophils and CD62P on platelets was also significantly inhibited by monoclonal antibody 166-32. This is consistent with the inhibition of the release of neutrophil-specific myeloperoxidase and elastase and platelet thrombospondin. The production of proinflammatory cytokine interleukin 8 was also suppressed by the antibody.

Conclusions: The alternative complement cascade is predominantly activated during extracorporeal circulation. Anti-factor D monoclonal antibody 166-32 is effective in inhibiting the activation of complement, neutrophils, and platelets. Inhibition of the alternative complement pathway by targeting factor D could be useful in reducing systemic inflammation in patients undergoing cardiopulmonary bypass.

From Tanox, Inc[a]; the Departments of Anesthesiology[b] and Surgery,[c] Baylor College of Medicine; Texas Children's Hospital[d]; and Texas Heart Institute,[e] Houston, Tex.

This study was supported by Tanox, Inc, Houston, Tex.

Received for publication Sept 18, 2000; revisions requested Dec 7, 2000; revisions received Jan 23, 2001; accepted for publication Jan 29, 2001.

Address for reprints: Michael Fung, PhD, Tanox, Inc, 10301 Stella Link, Houston, TX 77025 (E-mail: mfung@tanox.com).

*Current affiliation of Paul G. Loubser, MD: McAllen Medical Center, McAllen, Tex.

J Thorac Cardiovasc Surg 2001;122:113-22

Copyright © 2001 by The American Association for Thoracic Surgery

0022-5223/2001 $35.00 + 0 **12/1/114777**

doi:10.1067/mtc.2001.114777

Patients undergoing cardiopulmonary bypass (CPB) frequently manifest a generalized systemic inflammatory response syndrome, which may contribute to perioperative tissue injury and impaired hemostasis.[1-6] Serious inflammatory conditions could lead to delayed recovery and occasionally to multiorgan dysfunction.[1,2,7] Complement activation has been implicated as the most important cause of the

Samsung Bioepis Exhibit No. 1002
Page 923

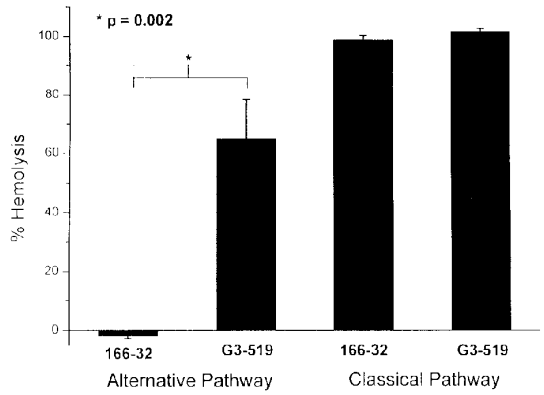


Figure 1. Selective inhibition of the alternative complement activity in human whole blood by MAb 166-32 at 18 μg/mL in the extracorporeal circulation studies before recirculation. MAb 166-32 did not inhibit the classical complement activity of the human plasma in the circuits. The negative control MAb G3-519 had no effects on either the alternative or the classical complement activity. Values are given as means ± SD from 5 paired experiments of the test and control circuits. Percentage of hemolysis was defined as indicated in the text. *P* values were determined by means of paired *t* tests between MAb 166-32– and MAb G3-519–treated circuits in the alternative complement hemolytic assays.

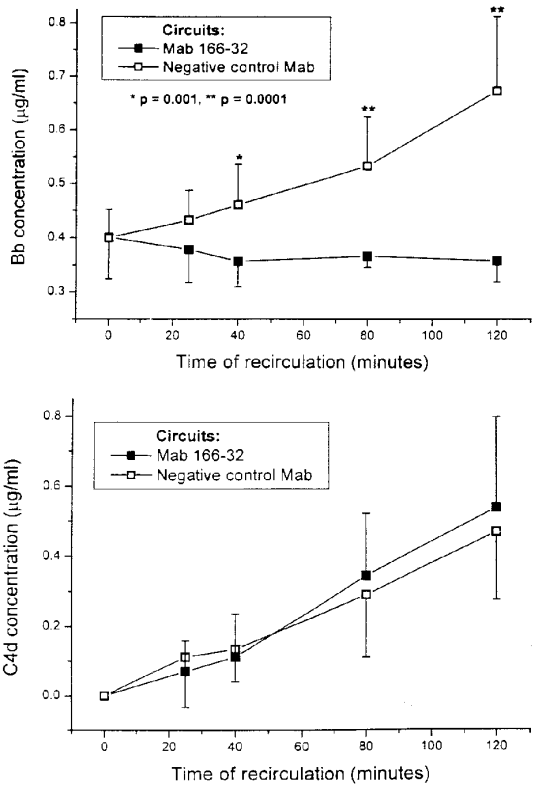


Figure 2. Bb and C4d production in the extracorporeal circuits treated with MAb 166-32 or the negative control MAb G3-519. Bb (A) and C4d (B) were measured by using a quantitative ELISA. Values are given as means ± SD from 5 paired experiments of the test and control circuits. MAb 166-32 completely inhibited the production of Bb, indicating complete suppression of the alternative complement pathway. *P* values were determined by 2-factor analysis of variance. There is no significant difference in the plasma levels of C4d between the test and control circuits as analyzed with 2-factor analysis of variance.

systemic inflammatory reaction.[1,3,4,6,8,9] It is attributed to the interaction between blood and the artificial surfaces of extracorporeal circuits constituting CPB machines.[10,11] Primary inflammatory substances are generated after activation of the complement system, including the anaphylatoxins C3a and C5a, the opsonin C3b, and the membrane attack complex C5b-9.[12] C3a and C5a are potent stimulators of neutrophils, monocytes, and platelets.[13-16] Activation of these cells results in release of proinflammatory cytokines (eg, interleukin 1 [IL-1], IL-6, IL-8, and tumor necrosis factor α), oxidative free radicals, and proteases.[17-22] C5a has been shown to upregulate adhesion molecules CD11b (α-integrin) and CD18 (β_2-integrin) of Mac-1 in polymorphonuclear cells (PMNs; comprising mainly neutrophils) and to induce degranulation of PMNs to release proinflammatory enzymes.[15,16,23-27] C5b-9 induces the expression of adhesion molecule P-selectin (CD62P) on platelets,[28] whereas both C5a and C5b-9 induce surface expression of P-selectin on endothelial cells.[29] These adhesion molecules are involved in the interaction among leukocytes, platelets, and endothelial cells. The expression of adhesion molecules on activated endothelial cells is responsible for sequestration of activated leukocytes, which then mediate tissue inflammation and injury.[24,29,30] In addition, C3a and C5a stimulate chemotaxis of human mast cells and trigger the release of histamine, proteases, and leukotrienes, which induce vascular permeability, vasoconstriction, and tissue inflammation.[31,32]

Factor D is the rate-limiting enzyme important for the activation and amplification of the alternative complement pathway.[33,34] Inasmuch as the concentration of factor D in human blood is among the lowest of all soluble complement components (approximately 2 μg/mL),[33] factor D is a potential target for inhibiting the activation of the alternative complement cascade associated with inflammatory responses. Previously, specific inhibition of the alternative complement pathway was demonstrated with monoclonal antibodies (MAbs) to human factor D with moderate activity in complement-mediated hemolytic assays.[35] Polyclonal rab-

Samsung Bioepis Exhibit No. 1002
Page 924

bit antibodies to mouse factor D were shown to inhibit alternative complement activity in mice.[36] We have generated a potent and specific MAb to human factor D, designated 166-32. The antibody inhibits human factor D at a molar ratio of about 1:2 in complement-mediated hemolytic assays.[37] The antibody was shown to protect isolated rabbit hearts from human complement-mediated injury, as manifested by an overall maintenance of myocardial function after challenge with human plasma.[37]

Recirculation of human whole blood in extracorporeal bypass circuits has been used extensively as a simulated model to study the activation of complement, leukocytes, and platelets in CPB.[15,16,28] The results of our extracorporeal circulation studies demonstrate that complete inhibition of the alternative complement cascade by anti-factor D MAb 166-32 suppresses activation of neutrophils and platelets, as well as inhibition of proinflammatory cytokine IL-8 production.

Methods

Design and Preparation of Extracorporeal Circuits

In the study whole blood was obtained from 5 healthy volunteers after approval by the Human Investigation Committee of Baylor College of Medicine. Informed consent was obtained from each participant. Whole blood from the same donor was used in both test and negative control circuits on the same day to avoid any variations in complement activity in donor blood.

Extracorporeal circuits were assembled by using a hollow-fiber pediatric membrane oxygenator with an integrated heat exchanger module (D 901 LILLPUT 1; DIDECO, Mirandola, Italy), a pediatric venous reservoir with an integrated cardiotomy filter (D 752 Venomidicard; DIDECO), a perfusion tubing set (Sorin Biomedical, Inc, Irvine, Calif), and a multiflow roller pump (Stöckert Instruments GmbH, Munich, Germany). Oxygenator and circuitry were primed with lactated Ringer's solution (Baxter Healthcare Corp, Deerfield, Ill). The prime fluid was warmed to 32°C with a cooler-heater (Sarns/3M Health Care, Ann Arbor, Mich) and circulated at 500 mL/min, and the sweep gas flow was maintained at 0.25 L/min with 100% oxygen. The sweep gas was changed to a mixture of oxygen (95%) and carbon dioxide (5%) after the blood was added to the circuit. The pH, P_{CO_2}, P_{O_2}, and perfusate temperature were continuously monitored throughout the recirculation period. Sodium bicarbonate was added as required to maintain pH in the range of 7.25 to 7.40.

Extracorporeal Circuit Operation and Blood Sampling

Blood (250 mL) was drawn over the course of 5 to 10 minutes into a transfer pack (Haemo-Pak; Chartermed, Inc, Lakewood, NJ) from healthy volunteers taking no medications. Before blood collection, porcine heparin (Elkins-Sinn, Cherry Hill, NJ) and anti-factor D MAb 166-32 (mouse immunoglobulin G_1) were added to a transfer pack at final blood concentrations of 5 U/mL and 18 μg/mL, respectively. This concentration of the antibody is equivalent to about 1.5 times the molar concentration of factor D in the blood. For the negative control, an isotype-matched irrelevant

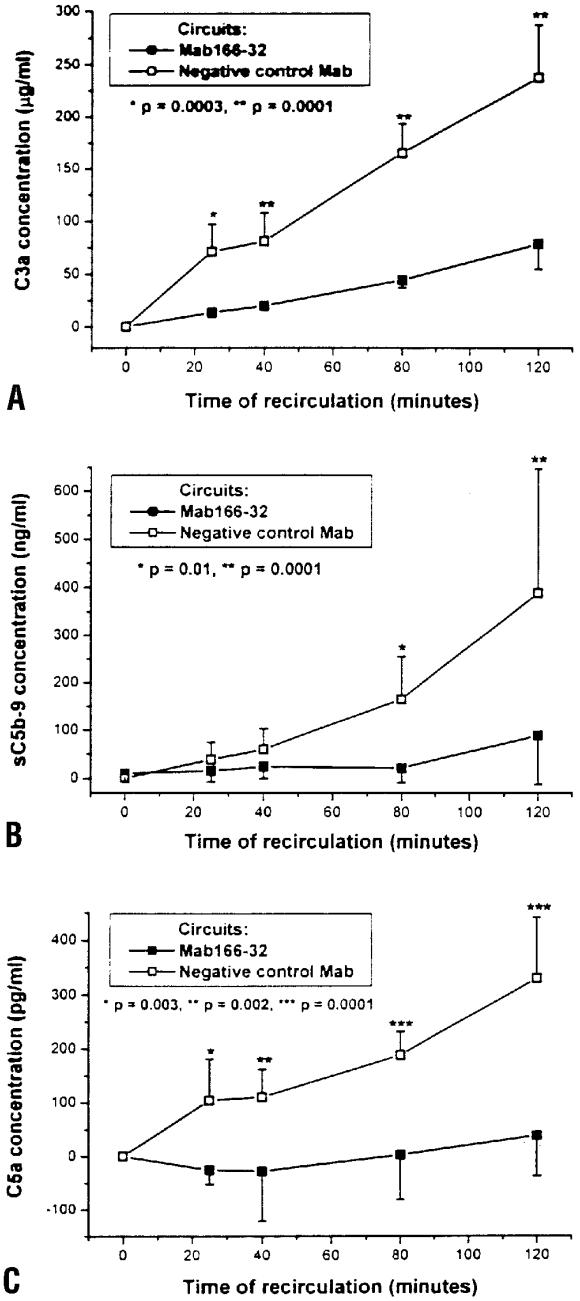


Figure 3. C3a, sC5b-9, and C5a production in the extracorporeal circuits treated with MAb 166-32 or the negative control MAb G3-519. C3a (A), sC5b-9 (B), and C5a (C) were measured by means of a quantitative ELISA. MAb 166-32 inhibited the production of C3a, sC5b-9, and C5a in the extracorporeal circuits compared with that in the MAb G3-519 circuits. Values are given as means ± SD from 5 paired experiments of the test and control circuits. *P* values were determined by 2-factor analysis of variance.

CPS

Samsung Bioepis Exhibit No. 1002
Page 925

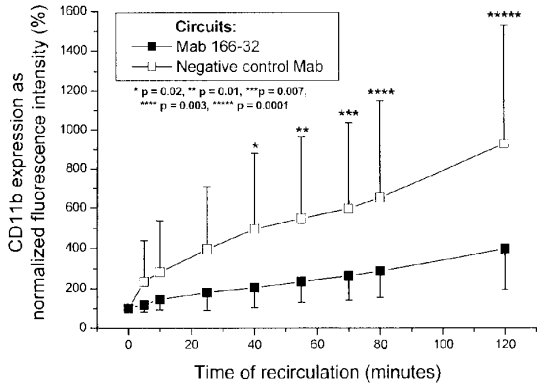


Figure 4. Inhibition of CD11b expression on PMNs in the extracorporeal circuits treated with MAb 166-32 compared with the circuits treated with the negative control MAb G3-519. The expression level of CD11b was measured by means of an immunofluorocytometric method and is expressed as the percentage of mean channel fluorescence intensity relative to the value at time 0 (100%). Values are given as means ± SD from 5 paired experiments of the test and control circuits. *P* values were determined by 2-factor analysis of variance.

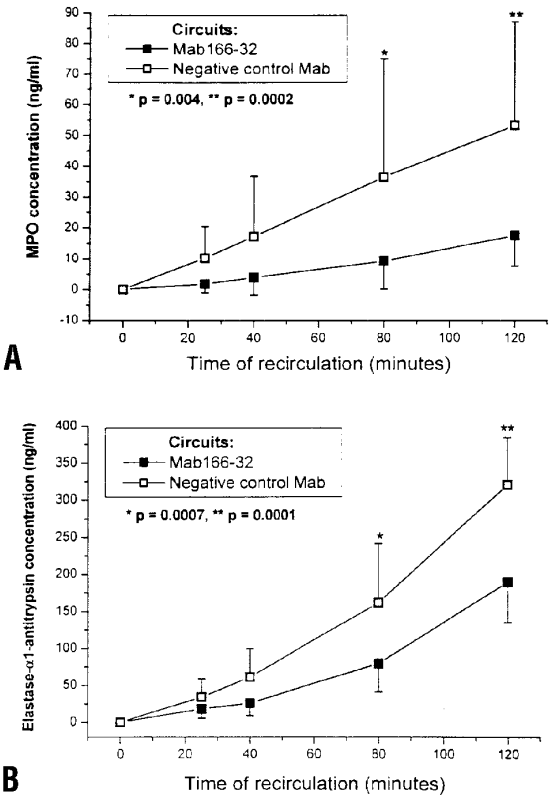


Figure 5. Inhibition of myeloperoxidase *(MPO)* and elastase release from neutrophils in the extracorporeal circuits treated with MAb 166-32 compared with that in the circuits treated with the negative control MAb G3-519. Myeloperoxidase (A) and elastase-α_1-antitrypsin inhibitor complex (B) were measured by a quantitative ELISA. Elastase released from activated neutrophils is quickly bound to α_1-antitrypsin inhibitor in the blood. Values are given as means ± SD from 5 paired experiments of the test and control circuits. *P* values were determined by 2-factor analysis of variance.

MAb (G3-519) was used at the same final concentration. MAb G3-519 (mouse immunoglobulin G_1) is specific to HIV-1 external envelope protein gp120. MAb G3-519 has previously been shown to have no effects on human complement activity.[37] The collected blood was then added to the reservoir through the prime port. Prime fluid was simultaneously withdrawn distal to the oxygenator outlet to yield a final circuit volume of 350 mL and a final hematocrit level of 25% to 28%. The pump flow rate was maintained at 500 mL/min, and the line pressure of the circuits was maintained at about 50 mm Hg throughout the entire experiment. Blood was recirculated with prime fluid, and complete mixing was accomplished within 3 minutes; a baseline sample was drawn and designated as time 0. In order to mimic the usual procedures of surgical operation under hypothermia, the circuit was then cooled to 27°C for 70 minutes, after which it was rewarmed to 37°C at 80 minutes. The temperature was maintained until 120 minutes, when the recirculation was terminated.

Blood samples were collected at 5, 10, 25, 40, 55, 70, 80, and 120 minutes during the recirculation. Plasma samples were prepared by immediate centrifugation at 2000*g* at 4°C. Aliquots of plasma were snap-frozen on dry ice and then stored at –80°C. The plasma samples were used for complement-mediated hemolytic assays and quantitation of neutrophil-specific myeloperoxidase and elastase, platelet thrombospondin, C5a, and IL-8. Aliquots of plasma for measurement of complement products C3a, C4d, sC5b-9, and Bb by means of enzyme-linked immunosorbent assay (ELISA) were immediately mixed with an equal volume of a specimen-stabilizing medium (Quidel, San Diego, Calif), snap-frozen on dry ice, and then stored at –80°C. Samples of whole blood were also collected for immunostaining of the activation cell-surface markers CD11b and CD62P on neutrophils and platelets, respectively. To prevent subsequent complement activation of the whole blood samples during the staining procedure, we added 10 μL of 1 mol/L ethylenediamine tetra-acetic acid to every milliliter of whole blood to give a final concentration of 10 mmol/L.

Complement-Mediated Hemolytic Assays

The alternative complement activity in the plasma samples from MAb 166-32–treated and MAb G3-519–treated circuits was measured with the use of rabbit red blood cells (RBCs). The plasma samples were treated with ethylene glycol-*bis*-(β-aminoethyl ether)-N,N,N′,N′-tetra-acetic acid (EGTA) at a final concentration of 50 mmol/L to chelate Ca^{++} for inhibition of the classical complement. Thirty microliters of rabbit RBC suspension (1.7×10^8 cells/mL) in gelatin/veronal-buffered saline solution (GVB/Mg-EGTA) containing 2 mmol/L $MgCl_2$ and 50 mmol/L EGTA were added to 100 μL of human plasma (final plasma concentration,

Samsung Bioepis Exhibit No. 1002
Page 926

77%). After incubation at 37°C for 30 minutes, the supernatants were collected, and optical density (OD) was read at 405 nm by using an ELISA plate reader. This OD reading was designated as OD_A. One hundred microliters of each plasma sample was mixed with 30 μL of GVB/Mg-EGTA to give the plasma color background (OD_B). For 100% hemolysis, 100 μL of water was mixed with 30 μL of rabbit RBC suspension. This OD reading was designated as OD_{100}. The percentage of hemolysis was determined by the following formula:

$$\text{Percentage hemolysis} = 100 \times (OD_A - OD_B)/OD_{100}$$

Spontaneous lysis of RBCs was negligible.

For classical pathway hemolysis, chicken RBCs (5×10^7 cells/mL) in GVB^{++} containing 0.5 mmol/L $MgCl_2$ and 0.15 mmol/L $CaCl_2$ were sensitized with purified antichicken RBC immunoglobulins at 8 μg/mL (InterCell Technologies, Hopewell, NJ) for 15 minutes at 4°C. The cells were then washed with GVB^{++}. The plasma samples were not treated with EGTA. The remaining procedures were identical to those for alternative pathway hemolysis described above.

Assays of Complement Activation Products

Plasma samples from the extracorporeal circuits were tested for the levels of Bb, C4d, C3a, and sC5b-9. Bb is a specific marker for the activation of the alternative complement pathway, whereas C4d is for the classical complement pathway. These substances were measured by means of commercially available quantitative ELISA kits (Quidel) according to the manufacturer's manuals.

Assays for Expression of CD11b on Neutrophils and CD62P on Platelets

The activation of neutrophils and platelets was quantitated by measuring the levels of the cell-surface expression of CD11b and CD62P on neutrophils and platelets, respectively. For CD11b labeling of neutrophils, 100 μL of whole blood collected from the circuits was immediately incubated with 20 μL of phycoerythrin-anti-CD11b antibody (Becton Dickinson, San Jose, Calif) for 10 minutes at room temperature. Then 1.4 mL of FACS Lysing solution (Becton Dickinson) was added for 10 minutes at room temperature to lyse RBCs and to fix leukocytes. The cells were then washed in phosphate-buffered saline solution and then fixed in 1% paraformaldehyde before analysis with an EPIC-XL flow cytometer (Coulter Corp, Miami, Fla). For double labeling to concomitantly identify the neutrophil population, fluorescein isothiocyanate (FITC)-anti-CD15 antibody (Becton Dickinson) was added for incubation together with phycoerythrin-anti-CD11b antibody.

For CD62P labeling of platelets, 40 μL of whole blood collected from the circuits was incubated with 20 μL of phycoerythrin-anti-CD62P antibody (Becton Dickinson) for 10 minutes at room temperature. Then the mixture was treated with FACS Lysing solution as described above. The platelets were washed in phosphate-buffered saline solution, fixed in 1% paraformaldehyde, and then analyzed as described above. For double labeling to concomitantly identify the platelet population, FITC-anti-CD41 antibody (Coulter) was added for incubation together with phycoerythrin-anti-CD62P antibody.

For flow cytometric measurement, PMN and platelet populations were identified by live gating on the basis of forward-scatter versus side-scatter parameters and specific staining with FITC-anti-CD15 antibody and FITC-anti-CD41 antibody, respectively. The background staining was gated by means of isotype-matched labeled antibodies. The intensity of expression of CD11b and CD62P was represented by the mean fluorescence intensity.

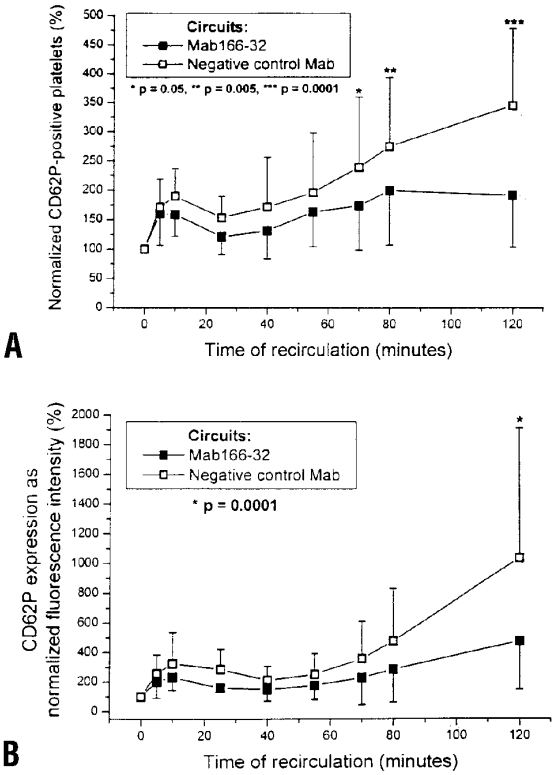


Figure 6. Inhibition of CD62P expression on platelets in the extracorporeal circuits treated with MAb 166-32 compared with the circuits treated with the negative control MAb G3-519. The percentages of $CD62P^+$ platelets (A) and the CD62P expression level in mean fluorescence intensity on platelets (B) are represented relative to the values at time 0 (100%). Values are given as means ± SD from 5 paired experiments of the test and control circuits. *P* values were determined by 2-factor analysis of variance.

Assay of C5a

C5a was measured quantitatively with an ELISA. Plasma samples were first treated with a precipitating reagent from a C5a radioimmunoassay kit (Amersham, Arlington Heights, Ill) to remove high-molecular-weight proteins, including C5. In the C5a ELISA purified rabbit anti-C5a antibodies (Calbiochem, La Jolla, Calif) were coated on wells of microtest plates to immobilize C5a in plasma samples. Purified sheep anti-human C5 antibodies (Biodesign, Kennebunk, Maine) were used to capture the immobilized C5a. The bound sheep antibodies were then detected by using horseradish peroxidase rabbit anti-sheep immunoglobulin G (Fc) antibodies (Jackson ImmunoResearch, West Grove, Pa). Peroxidase sub-

CPS

Samsung Bioepis Exhibit No. 1002
Page 927

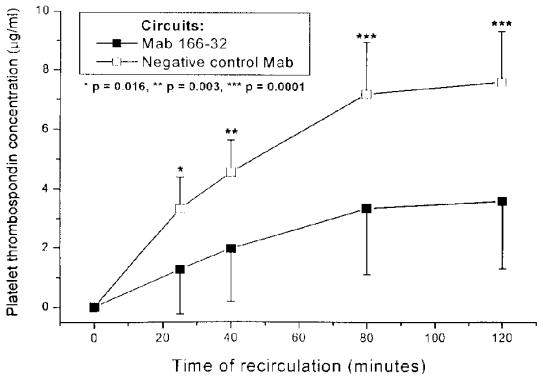


Figure 7. Inhibition of thrombospondin release from platelets in the extracorporeal circuits treated with MAb 166-32 compared with that in the circuits treated with the negative control MAb G3-519. Thrombospondin was measured by means of a quantitative ELISA. Values are given as means ± SD from 5 paired experiments of the test and control circuits. *P* values were determined by 2-factor analysis of variance.

strate solution was added for color development, and OD_{450} was measured by means of an ELISA plate reader. Purified C5a (Advanced Research Technologies, San Diego, Calif) was used as the standard for calibration.

Assay of Neutrophil-Specific Myeloperoxidase

Activation of neutrophils was also measured with an ELISA kit (R&D Systems, Inc, Minneapolis, Minn) to quantitate the amount of neutrophil-specific myeloperoxidase in the plasma samples from the extracorporeal circuits. The assays were performed according to the manufacturer's manual.

Assay of Elastase-α_1-Antitrypsin Inhibitor Complex

A quantitative ELISA was used to measure neutrophil-specific elastase-α_1-antitrypsin inhibitor complex, as described previously.[16] On activation, neutrophils release elastase, which is immediately complexed with α_1-antitrypsin inhibitor in the blood.[16] The assay used sheep anti-human neutrophil elastase polyclonal antibodies (Biodesign) to immobilize the complex and horseradish peroxidase–conjugated sheep anti-α_1-antitrypsin inhibitor polyclonal antibodies (Biodesign) for detection. As a calibration standard, elastase-α_1-antitrypsin inhibitor complex was made by mixing neutrophil-specific elastase (Elastin Products Company, Inc, Owensville, Mo) with α_1-antitrypsin inhibitor (Calbiochem).

Assay of Platelet Thrombospondin

A quantitative ELISA was used to measure platelet thrombospondin, which is an α-granule glycoprotein involved in platelet aggregation and is released on activation. In the assay thrombospondin was captured by using a pair of noncompetitive antithrombospondin MAbs, as described previously.[38] Monoclonal antithrombospondin antibody (clone P12, Coulter) was coated on wells of microtest plates to immobilize plasma thrombospondin. Biotinylated monoclonal antithrombospondin antibody (clone P10, Coulter) was used to capture immobilized thrombospondin. Purified thrombospondin (Calbiochem) was used as a calibration standard in the assay.

IL-8 ELISA

IL-8 was measured by using a quantitative ELISA kit (R&D Systems).

Statistical Analysis

The data from 5 paired circuits were statistically analyzed and represented as means ± SD. The data of hemolytic assays were analyzed with paired *t* tests. A 2-factor analysis of variance in a randomized block design was used to analyze the data from the other assays.

Results

Selective Inhibition of the Alternative Complement Activity by MAb 166-32

In the extracorporeal circulation study, MAb 166-32 was added to heparinized human whole blood at a final molar concentration of 1.5 times that of factor D in the blood. An isotype-matched negative control MAb, G3-519, was also studied at the same concentration in a parallel circuit by using whole blood from the same donor on the same day. The alternative and classical complement activities in the blood treated with the antibodies before recirculation were measured by means of hemolytic assays with rabbit RBCs and sensitized chicken RBCs, respectively. In the alternative pathway hemolytic assays, plasma from MAb G3-519–treated circuits at a final concentration of 77% induced lysis of rabbit RBCs by 65%, whereas no hemolysis was observed with plasma from MAb 166-32–treated circuits (Figure 1). In contrast, neither MAb 166-32 nor MAb G3-519 inhibited the lysis of sensitized chicken RBCs through the classical complement pathway (Figure 1). Therefore, MAb 166-32 is a selective and potent inhibitor of the alternative complement pathway.

Complement Activation in Extracorporeal Circuits

The selective inhibition of the alternative complement pathway by MAb 166-32 is in agreement with the complete inhibition of the production of Bb throughout the entire duration of recirculation (Figure 2, *A*). Bb is the enzymatic product of factor B on proteolysis by factor D, and therefore it is a specific marker for the activation of the alternative complement pathway. On the other hand, there was an increase in the plasma concentration of C4d in both circuits treated with either MAb 166-32 or MAb G3-519 (Figure 2, *B*). C4d is a specific marker for activation of the classical complement pathway.

The plasma concentrations of C3a, sC5b-9, and C5a in the extracorporeal circuits during recirculation were also measured. MAb 166-32 strongly inhibited the production of these substances (Figure 3, *A-C*). The formation of sC5b-9

Samsung Bioepis Exhibit No. 1002
Page 928

and C5a was completely inhibited during recirculation at hypothermic temperatures. At the end of 120 minutes of recirculation, MAb 166-32 inhibited the production of C3a, sC5b-9, and C5a by 67%, 77%, and 88%, respectively, compared with that of the negative control MAb G3-519. Because the alternative complement activity of the plasma in the test circuits was completely inhibited by MAb 166-32, the production of C3a, sC5b-9, and C5a in these circuits could be attributed to some activation of the classical pathway, as indicated by the elevation of plasma C4d levels (Figure 2, *B*).

Activation of Neutrophils in Extracorporeal Circuits

Activation of neutrophils as manifested by upregulation of surface expression of CD11b was measured by means of immunocytofluorometric analyses. The expression of CD11b on neutrophils in circuits treated with MAb 166-32 was significantly reduced compared with the negative control MAb G3-519 (Figure 4). At the end of 120 minutes of recirculation, MAb 166-32 inhibited CD11b expression on neutrophils by 57% compared with MAb G3-519.

The activation of neutrophils was also studied by measuring the levels of neutrophil-specific myeloperoxidase and elastase in plasma. The enzymes are stored in azurophilic primary granules of neutrophils and released on degranulation of activated neutrophils.[25-27] Consistent with the inhibition of neutrophil activation as manifested by the upregulation of CD11b expression (Figure 4), the release of both myeloperoxidase and elastase was also significantly inhibited by MAb 166-32 in the extracorporeal circuits compared with in the MAb G3-519–treated circuits (Figure 5, *A* and *B*). At the end of 120 minutes of recirculation, MAb 166-32 inhibited the release of myeloperoxidase and elastase by 67% and 41%, respectively, compared with MAb G3-519.

Activation of Platelets in Extracorporeal Circuits

The effects of MAb 166-32 on platelet activation during recirculation in extracorporeal circuits were examined. The number of $CD62P^+$ platelets and the level of CD62P expression were significantly reduced in MAb 166-32–treated circuits compared with in MAb G3-519–treated circuits (Figure 6, *A* and *B*). At the end of 120 minutes of recirculation, the number of $CD62^+$ platelets and the level of CD62 expression were reduced by 45% and 54%, respectively, in MAb 166-32–treated circuits compared with in MAb G3-519–treated circuits. These results corroborate with the inhibition of thrombospondin release from activated platelets by MAb 166-32 treatment by 53% at the end of 120 minutes of recirculation compared with MAb G3-519 (Figure 7).

Production of IL-8 in Extracorporeal Circuits

To further examine the effects of complement inhibition on other inflammatory responses in whole blood during recirculation, we examined the production of the proinflammatory cytokine IL-8 in the test and control extracorporeal circuits. IL-8 is produced predominantly by activated neutrophils, monocytes-macrophages, and T cells. During the recirculation at hypothermic temperatures, the plasma levels of IL-8 did not increase, even in MAb G3-519–treated circuits. However, the plasma levels of IL-8 increased dramatically when the circuits were warmed to 37°C. At the end of 120 minutes of recirculation, IL-8 production in MAb 166-32–treated circuits was significantly reduced by 73% compared with MAb G3-519–treated circuits (Figure 8).

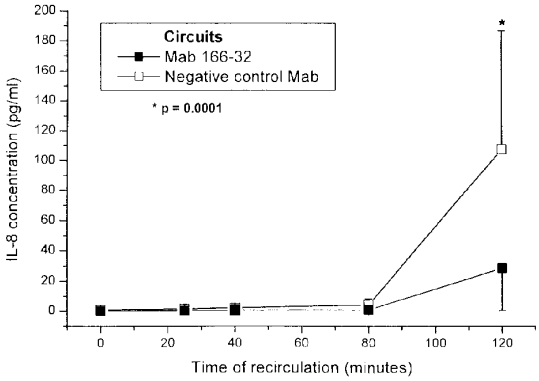


Figure 8. Inhibition of IL-8 production in the extracorporeal circuits treated with MAb 166-32 compared with that in the circuits treated with the negative control MAb G3-519. IL-8 was measured by a quantitative ELISA. Values are given as means ± SD from 5 paired experiments of the test and control circuits. *P* values were determined by 2-factor analysis of variance.

Discussion

The complement cascade is activated predominantly through the alternative or the classical pathway.[12] Activation of the alternative complement pathway is induced through the direct activation of C3, whereas the classical complement pathway is activated through the initial activation of C1. Recent studies show that complement can also be activated through the lectin pathway, which involves the initial binding of mannose-binding lectin and the subsequent activation of C2 and C4, which are common to the classical complement pathway.[39,40] Interestingly, accumulating evidence indicates that the alternative complement pathway participates in the amplification of the activity of both the classical complement and the lectin pathways as a result of C3b deposition, which triggers the formation of the alternative C3/C5 convertases.[40,41] Therefore, the alternative complement pathway may play a broad role in inflammation resulting from complement activation.

In CPB circuits the alternative complement pathway plays a predominant role in complement activation, result-

CPS

Samsung Bioepis Exhibit No. 1002
Page 929

ing from the interaction of blood with the artificial surfaces of CPB circuits.[1,6,11] However, natural human antibodies against dialysis membranes have been described, and it is possible that natural antibodies against components of the CPB circuits or membrane could be responsible for the C4d production in our study.[42] Interestingly, the alternative complement pathway was shown to play a crucial role in the amplification of the classical complement pathway once it was initiated.[42] In addition, activation of the classical complement pathway in CPB circuits could also be triggered by means of contact activation of the intrinsic coagulation pathway.[10,43,44] As a result, factor XIIa, factor XIa, and kallikrein bind C1 esterase inhibitor, an endogenous inhibitor of activated C1. When the plasma concentration of C1 esterase inhibitor is diminished, C1 becomes more susceptible to activation, and thus the classical complement pathway is augmented. Inasmuch as the production of C5a and sC5b-9 was substantially inhibited compared with that of C3a in our study, the moderate activation of the classical pathway as indicated by the formation of C4d during hypothermic recirculation does not seem to effectively activate C5. Similarly, although protamine neutralization of heparin after CPB causes activation of the classical complement pathway,[6,45] the activation of C5 by protamine-heparin complexes is found to be ineffective.[6,8] Using anti-factor D MAb 166-32 to selectively inhibit the alternative complement pathway, we confirm the previous findings that the alternative complement pathway is predominantly activated in CPB circuits.[1,6,8]

The results from our study show that anti-factor D MAb 166-32 is a potent and specific inhibitor of the alternative complement pathway. At a concentration (18 μg/mL) equivalent to 1.5 times the molar concentration of factor D in human blood, MAb 166-32 completely inhibits the alternative complement activity, as evidenced by the complete blockade of Bb formation in the CPB circuits. Inhibition of the alternative complement pathway by anti-factor D MAb 166-32 suppresses the production of C3a, sC5b-9, and C5a. These substances are known to activate neutrophils, monocytes, platelets, mast cells, and endothelial cells.[13-16,28,29] Reduction of CD11 (α-integrin) expression on neutrophils prevents their binding to platelets and endothelial cells through the interaction with CD62P on these cells.[46,47] Sequestration of activated neutrophils on endothelial cells can result in tissue inflammation and injury.[24,29,30] It has been shown that CD11b expression on monocytes is increased by C3a but not C5a and sC5b-9 in CPB circuits.[15,16] Therefore, the reduction of C3a production by MAb 166-32 in our study could be important for controlling monocyte adhesion. Although the exact roles of activated monocytes in the inflammatory response of CPB have yet to be delineated, it is likely that proinflammatory cytokine release and tissue factor–mediated thrombosis are involved.[13,48] Because CR3 (Mac-1; CD11/CD18) on leukocytes can bind to iC3b on complement-activated surfaces in CPB circuits,[49] this could contribute to the depletion of leukocytes commonly observed in patients undergoing CPB.[15,44] Inhibition of CD11b upregulation by MAb 166-32 in our study could prevent the loss of leukocytes during extracorporeal circulation.

The results from our study also show that inhibition of the alternative complement pathway by anti-factor D MAb 166-32 suppresses the degranulation of neutrophils, as indicated by the reduced release of myelopoxidase and elastase. Myeloperoxidase is responsible for production of hypochlorous acid, a potent oxidant that could cause tissue damage, whereas elastase could mediate tissue injury as a result of hydrolysis of elastin in extracellular matrices.[25-27] The incomplete inhibition of neutrophil degranulation by anti-factor D MAb 166-32 in this study might be attributed to contact activation, which results in the production of kallikrein, a mediator that is known to activate neutrophils to release elastase.[50] Similarly, platelets could be activated by thrombin as a result of initiation of the intrinsic coagulation pathway after contact activation.[51]

IL-8 is closely associated with inflammatory diseases and CPB.[19-22] Our results show that anti-factor D MAb reduces the release of IL-8. IL-8 causes chemotaxis of neutrophils, T cells, and basophils; degranulation of neutrophils; and adhesion of neutrophils to endothelial cells.[30] Therefore, the reduction of IL-8 production could reduce multiple inflammatory responses observed in CPB. Delayed increase of plasma IL-8 in our extracorporeal circulation study is consistent with the observation in operations involving CPB.[19,21,22]

One limitation of the simulated extracorporeal circulation model of CPB used in this study is the inability to study the inflammatory responses of endothelial cells caused by complement activation. In this regard, both C5a and C5b-9 were shown to induce surface expression of P-selectin on endothelial cells.[29]

Several complement inhibitors are being studied for potential applications in CPB. They include a recombinant soluble complement receptor 1 (sCR1),[52] a humanized single-chain anti-C5 antibody (h5G1.1-scFv),[53] a recombinant fusion hybrid (CAB-2) of human membrane cofactor protein and human decay accelerating factor,[16] and a 13-residue C3-binding cyclic peptide (Compstatin).[54] sCR1 and CAB-2 inhibit the classical and alternative complement pathways at the steps of C3 and C5 activation. Compstatin inhibits both complement pathways at the step of C3 activation, whereas h5G1.1-scFv does so only at the step of C5 activation. The results of our study strongly indicate that the alternative complement activation plays an important role in inducing a plethora of cellular and humoral inflammatory responses in CPB. Anti-factor D MAb 166-32 is effective in

Samsung Bioepis Exhibit No. 1002
Page 930

inhibiting the activation of C3 and C5 and the other inflammatory responses in extracorporeal circulation. Therefore, complete inhibition of the alternative complement pathway by targeting factor D could be useful to reduce systemic inflammation in patients undergoing CPB.

We thank the volunteers for blood donation; the General Clinical Research Center of Texas Children's Hospital (supported by National Institute of Health grant RR00188) for excellent assistance; Kenneth Pinkston, BS, of Tanox, Inc, for valuable suggestions; and Mary Claire McGarry, CCP, of the Texas Children's Hospital and Joyce Bigley, CCP, of the Texas Heart Institute for excellent technical assistance.

References

1. Kirklin JK, Westaby S, Blackstone EH, Kirklin JW, Chenoweth DE, Pacifico AD. Complement and the damaging effects of cardiopulmonary bypass. *J Thorac Cardiovasc Surg.* 1983;86:845-57.
2. Westaby S. Organ dysfunction after cardiopulmonary bypass: a systemic inflammatory reaction initiated by the extracorporeal circuit. *Intensive Care Med.* 1987;13:89-95.
3. Seghaye MC, Duchateau J, Grabitz RG. Complement activation during cardiopulmonary bypass in infants and children: relation to postoperative multiple system organ failure. *J Thorac Cardiovasc Surg.* 1993;106:978-87.
4. Asimakopoulos G. Mechanisms of the systemic inflammatory response. *Perfusion.* 1999;14:269-77.
5. Nieman G, Searle B, Carney D, McCann U, Schiller H, Lutz C, et al. Systemic inflammation induced by cardiopulmonary bypass: a review of pathogenesis and treatment. *J Extracorp Technol.* 1999;31:202-10.
6. Kirklin JK, Chenoweth DE, Naftel DC, Blackstone EH, Kirklin JW, Bitran DD, et al. Effects of protamine administration after cardiopulmonary bypass on complement, blood elements, and hemodynamic state. *Ann Thorac Surg.* 1986;41:193-9.
7. Taylor KM. Central nervous system effects of cardiopulmonary bypass. *Ann Thorac Surg.* 1998;66:S20-4.
8. Fosse E, Mollnes TE, Ingvalden B. Complement activation during major operations with or without cardiopulmonary bypass. *J Thorac Cardiovasc Surg.* 1987;93:860-6.
9. Gu J, Mariani MA, Boonstra PW, Grandjean JG, van Oeveren W. Complement activation in coronary artery bypass grafting patients without cardiopulmonary bypass. *Chest.* 1999;116:892-8.
10. Mollnes TE. Complement and biocompatibility. *Vox Sang.* 1998;74(Suppl 2):303-7.
11. Vallhonrat H, Swinford RD, Ingelfinger JR, Williams WW, Ryan DO, Tolkoff-Rubin N, et al. Rapid activation of the alternative pathway of complement by extracorporeal membrane oxygenation. *ASIAO J.* 1999;45:113-4.
12. Holers VM. Complement. In: Rich RR, Fleisher TA, Schwartz BD, Shearer WT, Strober W, editors. Clinical immunology: principles and practice. St Louis: Mosby; 1996. p. 363-91.
13. Haeffner-Cavaillon N, Cavaillon JM, Laude M, Kazatchkine MD. C3a(C3adesArg) induces production and release of interleukin 1 by cultured human monocytes. *J Immunol.* 1987;139:794-9.
14. Fletcher MP, Stahl G, Longhurst J. C5a-induced myocardial ischemia: role for CD18-dependent PMN localization and PMN-platelet interactions. *Am J Physiol.* 1993;265:H1750-61.
15. Rinder CS, Rinder HM, Smith BR, Fitch JCK, Smith MJ, Tracey JB, et al. Blockade of C5a and C5b-9 generation inhibits leukocyte and platelet activation during extracorporeal circulation. *J Clin Invest.* 1995;96:1564-72.
16. Rinder CS, Rinder HM, Johnson K, Smith M, Lee DL, Tracey J, et al. Role of C3 cleavage in monocyte activation during extracorporeal circulation. *Circulation.* 1999;100:553-8.
17. Schindler R, Gelfand JA, Dinarello CA. Recombinant C5a stimulates transcription rather than translation of IL-1 and tumor necrosis factor: translational signal provided by lipopolysaccharide or IL-1 itself. *Blood.* 1990;76:1631-8.
18. Cruickshank AM, Fraser WD, Burns HJG. Response of serum interleukin-6 in patients undergoing elective surgery of varying severity. *Clin Sci.* 1990;79:161-5.
19. Kawamura T, Wakusawa R, Okada K. Elevation of cyokines during open heart surgery with CPB: participation of interleukin-8 and 6 in reperfusion injury. *Can J Anaesth.* 1993;40:1016-21.
20. Steinberg JB, Kapelanski DP, Olson JD. Cytokine and complement levels in patients undergoing cardiopulmonary bypass. *J Thorac Cardiovasc Surg.* 1993;106:1008-16.
21. Finn A, Naik S, Klein N. Interleukin-8 release and neutrophil degranulation after pediatric cardiopulmonary bypass. *J Thorac Cardiovasc Surg.* 1995;110:99-102.
22. Ashraf SS, Tian Y, Cowan D, Shaikh R, Parsloe M, Martin P, et al. Proinflammatory cytokine release during pediatric cardiopulmonary bypass: influence of centrifugal and roller pumps. *J Cardiothorac Vasc Anesth.* 1997;11:718-22.
23. Rinder C, Fitch J. Amplification of the inflammatory response: adhesion molecules associated with platelet/white cell responses. *J Cardiovasc Pharmacol.* 1996;27:S6-12.
24. Evangelista V, Manarini S, Sideri R, Rotondo S, Marttelli, N, Piccoli A, et al. Platelet/polymorphonuclear leukocyte interaction: P-selectin triggers protein-tyrosine kinase phosphorylation-dependent CD11b/CD18 adhesion: role of PSGL-1 as signaling molecule. *Blood.* 1999;93:876-85.
25. Kinkade JM Jr, Pember SO, Barnes KC, Shapira R, Spitznagel JK, Martin LE. Differential distribution of distinct forms of myeloperoxidase in different azurophilic granule subpopulations from human neutrophils. *Biochem Biophys Res Commun.* 1983;114:296-303.
26. Lamb NJ, Gutteridge JM, Baker C, Evans TW, Quinlan GJ. Oxidative damage to proteins of bronchoalveolar lavage fluid in patients with acute respiratory distress syndrome: evidence for neutrophil-mediated hydroxylation, nitration, and chlorination. *Crit Care Med.* 1999;27:1738-44.
27. Fujie K, Shinguh Y, Inamura N, Yasumitsu R, Okamoto M, Okuhara M. Release of neutrophil elastase and its role in tissue injury in acute inflammation: effect of the elastase inhibitor, FR134043. *Eur J Pharmacol.* 1999;374:117-25.
28. Rinder CS, Rinder HM, Smith MJ, Tracey JB, Fich J, Li L, et al. Selective blockade of membrane attack complex formation during simulated extracorporeal circulation inhibits platelet but not leukocyte activation. *J Thorac Cardiovasc Surg.* 1999;118:460-6.
29. Foreman KE, Vaporciyan AA, Bonish BK, Jones ML, Johnson KJ, Glovsky MM, et al. C5a-induced expression of P-selectin in endothelial cells. *J Clin Invest.* 1994;94:1147-55.
30. Lentsch AB, Ward PA. Regulation of inflammatory vascular damage. J Pathol. 2000;190:343-8.
31. Hartmann K, Henz BM, Kruger-Krasagakes S, Kohl J, Burger R, Guhl S, et al. C3a and C5a stimulate chemotaxis of human mast cells. *Blood.* 1997;89:2863-70.
32. Fukuoka Y, Hugli TE. Anaphylatoxin binding and degradation by rat peritoneal mast cells: mechanisms of degranulation and control. *J Immunol.* 1990;145:1851-8.
33. Lesavre PH, Müller-Eberhard HJ. Mechanism of action of factor D of the alternative complement pathway. *J Exp Med.* 1978;148:1498-509.
34. Volanakis JE, Narayana SVL. Complement factor D, a novel serine protease. *Protein Sci.* 1996;5:553-64.
35. Pascual M, Catana E, Spertini F, Macon K, Volanakis JE, Schifferli JA. A monoclonal antibody which blocks the function of factor D of human complement. *J Immunol Methods.* 1990;127:263-9.
36. Pascual M, Catana E, White T, Spiegelman BM, Schifferli JA. Inhibition of complement alternative pathway in mice with Fab antibody to recombinant adipsin/factor D. *Eur J Immunol.* 1993;23:1389-92.
37. Tanehco EJ, Kilgore KE, Liff DA, Murphy KL, Fung MS, Sun WN, et al. The anti-factor D antibody, MAb 166-32, inhibits the alternative pathway of the human complement system. *Transplant Proc.* 1999;31:2168-71.
38. Fiane AE, Videm V, Johansen HT, Mellbye OJ, Nielsen EW, Mollnes TE. C1-inhibitor attenuates hyperacute rejection and inhibits comple-

Samsung Bioepis Exhibit No. 1002
Page 931

ment, leukocyte and platelet activation in an ex vivo pig-to-human perfusion model. *Immunopharmacology.* 1999;42:231-43.
39. Matsushita M, Fujita T. Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like serine protease. *J Exp Med.* 1992;176:1497-502.
40. Suankratay C, Zhang X-H, Zhang Y, Lint TF, Gewurz H. Requirement for the alternative pathway as well as C4 and C2 in complement-dependent hemolysis via the lectin pathway. *J Immunol.* 1998;160:3006-13.
41. Farries TC, Steur KLK, Atkinson JP. The mechanism of activation of the alternative pathway of complement by cell-bound C4b. *Mol Immunol.* 1990;27:1155-61.
42. Lhotta K, Wurzner R, Kronenberg F, Oppermann M, Konig P. Rapid activation of the complement system by cuprophane depends on complement component C4. *Kidney Int.* 1998;53:1044-51.
43. Levy JH. Complement and contact activation. In: Levy JH, editor. Anaphylactic reactions in anesthesia and intensive care. Boston: Butterworth-Heinemann; 1992. p. 51-62.
44. Edmunds LH Jr. Inflammatory response to cardiopulmonary bypass. *Ann Thorac Surg.* 1998;66:S12-6.
45. Carr JA, Silverman N. The heparin-protamine interaction: a review. *J Cardiovasc Surg* (Torino). 1999;40:659-66.
46. Geng JG, Bevilacqua MP, Moore KL, McIntyre TM, Prescott SM, Kim JM, et al. Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. *Nature.* 1990;343:757-61.
47. Hamburger SA, McEver RP. GMP-140 mediates adhesion of stimulated platelets to neutrophils. *Blood.* 1990;75:550-5.
48. Kappelmayer J, Bernabei A, Edmunds LH Jr, Edgington TS, Colman RW. Tissue factor is expressed on monocytes during simulated extracorporeal circulation. *Circ Res.* 1993;72:1075-81.
49. Pekna M, Nilsson L, Nilsson EK, Nilsson UR, Nilsson B. Evidence for iC3 generation during cardiopulmonary bypass as the result of blood-gas interaction. *Clin Exp Immunol.* 1993;91:404-9.
50. Wachtfogel YT, Hack CE, Nuijens JH, Kettner C, Reilly TM, Knabb RN, et al. Selective kallikrein inhibitors alter human neutrophil elastase release during extracorporeal circulation. *J Physiol.* 1995;268:H1352-7.
51. Wachtfogel YT, Kettner C, Hack CE, Nuijens JH, Reilly TM, Knabb RM, et al. Thrombin and human plasma kallikrein inhibition during simulated extracorporeal circulation block platelet and neutrophil activation. *Thromb Haemost.* 1998;80:686-91.
52. Chai PJ, Nassar R, Oakeley AE, Craig DM, Quick G Jr, Jaggers J, et al. Soluble complement receptor-1 protects heart, lung, and cardiac myofilament function from cardiopulmonary bypass damage. *Circulation.* 2000;101:541-6.
53. Fitch JCK, Rollins S, Matis L, Alford B, Aranki S, Collard CD, et al. Pharmacology and biological efficacy of a recombinant, humanized, single-chain antibody C5 complement inhibitor in patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass. *Circulation.* 1999;100:2499-506.
54. Nilsson B, Larsson R, Hong J, Elgue G, Ekdahl KN, Sahu A, et al. Compstatin inhibits complement and cellular activation in whole blood in two models of extracorporeal circulation. *Blood.* 1998;92:1661-7.

CPS

Availability of Journal back issues

As a service to our subscribers, copies of back issues of *The Journal of Thoracic and Cardiovascular Surgery* for the preceding 5 years are maintained and are available for purchase from Mosby until inventory is depleted. The following quantity discounts are available: 25% off on quantities of 12 to 23, and one third off on quantities of 24 or more. Please write to Mosby, Subscription Customer Service, 6277 Sea Harbor Dr, Orlando, FL 32877, or call 800-654-2452 or 407-345-4000 for information on availability of particular issues and prices. If unavailable from the publisher, photocopies of complete issues may be purchased from Bell & Howell Information and Learning, 300 N Zeeb Rd, Ann Arbor, MI 48106-1346; 734-761-4700 or 800-521-0600.

Samsung Bioepis Exhibit No. 1002
Page 932

Immunity, Vol. 20, 517–527, May, 2004, Copyright ©2004 by Cell Press

Pathogenic Roles of B Cells in Human Autoimmunity: Insights from the Clinic

Review

Flavius Martin[1,*] and Andrew C. Chan[1,2,*]
[1]Department of Immunology
[2]Department of Antibody Engineering
Genentech, Inc.
One DNA Way, MS-34
South San Francisco, California 94080

The pathogenic roles of B cells in human autoimmune diseases involve a multitude of mechanistic pathways and include the well-established contributions of autoantibodies and immune complexes that induce local inflammatory reactions and tissue destruction. Recent results using several novel B cell-directed therapies have provided new insights into additional roles of B cells in human autoimmunity. In this review, we will highlight some of these studies and discuss how clinical insights parallel murine models of normal immunity and autoimmunity.

Treatment of autoimmune disorders has begun a transformation from an armamentarium of nonspecific therapies that affect a wide range of cells and signaling pathways to targeted therapies that modulate specific cytokines (e.g., tumor necrosis factor and IL-1), cell types (e.g., $CD3^+$ T cells and $CD20^+$ B cells), or inhibit a unique signaling pathway (Kamradt and Mitchison, 2001; Davidson and Diamond, 2001; Keystone, 2003; Gottlieb, 2003; Shanahan et al., 2003). The advent of targeted therapies in the treatment of human autoimmune diseases has not only significantly improved patients' lives and changed clinical therapeutic paradigms but has also led a renaissance in understanding pathogenesis of human disorders (Anasetti et al., 1992; Weinblatt et al., 1999; Lipsky et al., 2000; Pisetsky, 2000b; Cohen et al., 2002; Kremer et al., 2003; Keystone et al., 2003; Edwards et al., 2004). Our insights into the pathogenic roles of B cell dysfunction in human autoimmune diseases circa 2004 remain limited. While prophylactic or interventional preclinical studies in rodent models of human diseases are enlightening, many of these genetically homogeneous models do not translate well into the heterogeneous nature of human biology and diseases. Hence, a challenge to drug discovery as well as human immunology is to understand how probing B cell biology in human autoimmunity translates back to these preclinical models of human disease and then applying this new knowledge to advance patient care. In this discussion, we will review how therapies in human autoimmune diseases have provided insights into our expanding understanding of B cell functions in human autoimmune disorders (Figure 1).

The scientific routes of B cell modulation began with the elegant surgical approaches in birds and underscored the importance of the Bursa of Fabricius in B cell ontogeny (Cooper et al., 1965). This was followed by pioneering work using anti-immunoglobulin (Ig) reagents in which pre- and perinatal treatment of mice with anti-Ig polyclonal reagents successfully depleted surface positive B lineage cells (Cooper et al., 1980). Such a strategy was not applicable to adult animals that have high levels of circulating Igs that function as a sink for the depleting anti-Ig reagents. Attempts to translate these observations into clinical use for B cell lymphomas focused initially around the idea of anti-idiotype (Id) antibodies passively delivered or induced through active immunization (anti-Id vaccines) (McCarthy et al., 2003). Although this approach seemed ideal from a scientific perspective, the preexisting suppressed immune systems of cancer patients, phenomenon of tumor escape, as well as manufacturing related factors have delayed the translation of these therapies to clinical practice. The alternative approach of using antibodies directed against pan-B cell targets was faster to reach fruition through the development of antibodies targeting the CD20, CD22, and CD52 surface molecules (Treon and Anderson, 2000; Juweid, 2003; Cersosimo, 2003; Robak, 2004). All these approaches rely on the ability of passively infused antibodies to deplete antigen-bearing cells. This depletion is temporary and ceases through the continuous repopulation of the B lineage from precursors residing within primary hematopoietic organs.

In contrast to the B cell malignancies, non-Hodgkin's lymphoma, and chronic lymphocytic leukemia, in which one or more proliferating B cell clones can be identified at a cellular and molecular level, the identity and specificity of the pathogenic B cell clones in autoimmune diseases are rarely known. In a subset of lymphoma and leukemia patients, the malignant B cell clones produce low-affinity paraproteins (antibodies) that manifest autoimmune paraneoplastic syndromes. These paraproteins bind a variety of self-antigens, including those expressed on red blood cells (RBCs) that result in autoimmune hemolytic anemia (e.g., IgM cold agglutinin disease), or C1 esterase complement inhibitor that manifests clinically with angioedema (Silberstein, 1994; Pascual et al., 1997; Ward, 2001). Treatment of the underlying lymphoma or leukemia and eradication of the malignant B cell clone improves the associated clinical autoimmune manifestations. For most autoimmune diseases, however, the mechanistic relationship between pathogenic B cells, their products (e.g., autoantibodies), and clinical manifestations remains elusive. Nonetheless, the emerging findings suggest that B lineage depletion or modulation might be beneficial and provides a basis to further explore the role of B lineage function in human biology.

Autoantibodies—Diagnosis and Pathogenesis

Autoantibodies have served historically as the sine qua non to indicate a role for B cells in autoimmune diseases. They provide diagnostic and prognostic criteria, play a requisite role in disease pathogenesis, and serve as surrogate markers for disease activity.

The first category, while not mutually exclusive with the others, represents a large proportion of autoantibodies and typically is not thought to play a role in disease

*Correspondence: flavius@gene.com (F.M.); acc@gene.com (A.C.C.)

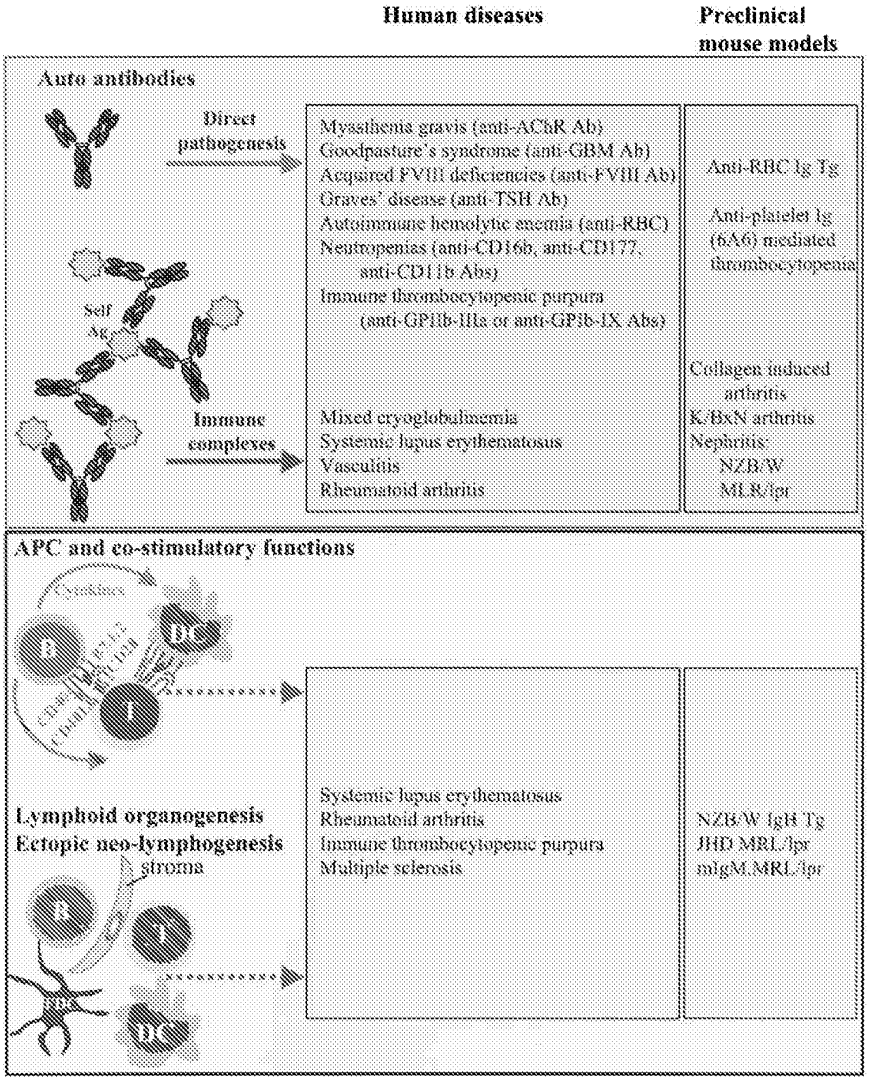


Figure 1. Effector Mechanisms of Human and Mouse B Cell Autoimmunity

"Classic" antibody-dependent mechanisms are responsible either directly or through immune complexes for the pathogenesis of specific autoimmune diseases. Several other mechanisms, including antigen presentation (APC), costimulatory functions (surface molecules and secreted cytokines), and the ability to support de novo lymphoid tissue organization (neolymphogenesis) may explain other parts of B cell involvement in complex autoimmune conditions.

AchR, acetylcholine receptor; GMB, glomerular basement membrane; FVIII, factor VIII; TSH, thyroid-stimulating hormone; RBC, red blood cells; GPIIb-IIIa, glycoprotein IIb-IIIa; GPIb-IX, glycoprotein Ib-IX; Ag, antigen; B, B cell; T, T cell; FDC, follicular dendritic cell; DC, dendritic cell.

pathogenesis. However, the latter may merely reflect our lack of understanding of antigen specificity and its role in disease pathogenesis. Nonetheless, many antibodies provide highly sensitive and/or specific diagnostic criteria for the presence of a specific clinical syndrome. For example, the presence of anti-nuclear antibodies (ANA) serves as an extremely sensitive diagnostic test for systemic lupus erythematosus (SLE). This autoimmune disorder is characterized by T, B, and myeloid cell dysregulation that gives rise to immune complex (IC)-mediated deposition and inflammatory damage in a variety of organs, including skin, lung, kidney, blood vessels, and the nervous system (Pisetsky, 2000a). However, this serologic marker is observed in a variety of other autoimmune disorders, and the level of serum ANAs does not correlate with the severity of clinical disease. Patients can have persistently high ANA titers despite the absence of clinical disease. Conversely, patients experience disease flares without any alterations in ANA levels. Other types of autoantibodies in SLE patients can also be used to aid diagnosis and prognostication of patient subsets—the presence of

anti-Sm antibodies is highly specific for SLE diagnosis, anti-RNP antibodies identify a subset of patients with a different clinical course of mixed connective tissue disease, anti-phospholipid antibodies are associated with an increased risk of thrombotic events, anti-ribosomal P antibodies identify patients with increased risk of central nervous system disease, and anti-Ro (SSA) antibodies are causally linked with an increased risk for the development of complete heart block (CHB) in the newborns of SLE patients (Ruiz-Irastorza et al., 2002; Reichlin, 2003; Buyon and Clancy, 2003). In the case of anti-Ro antibodies, only 50% of women have symptomatic SLE or Sjogren's syndrome at the time their babies are born with CHB. Yet, a large proportion of these asymptomatic women ultimately develop clinical SLE and Sjogren's disease over the ensuing decade (Brucato et al., 2002).

Aside from rheumatologic disorders, examples of pathogenic autoantibodies include the anti-acetylcholine receptor (AChR) and anti-glomerular basement membrane (GBM) antibodies in myasthenia gravis and Goodpasture's syndrome, respectively. Anti-AChR antibodies alter AChR organization on the postsynaptic neuronal plate and functionally block acetylcholine neurotransmission to induce muscle paralysis, in particular impairment of diaphragmatic function, with progression to respiratory failure and death (Vincent, 2002). The histological demonstration of these complement fixing antibodies on myelinated fibers from affected patients and ability of plasma exchange and intravenous immunoglobulin (IVIG) to improve clinical course together support a pathogenic role for anti-AChR antibodies in MG (Richman and Agius, 2003). Binding of anti-GBM antibodies to the $\alpha 3$ chain of type IV collagen in the lung and kidneys results in pulmonary hemorrhage and glomerulonephritis, respectively, the latter of which can progress to renal failure. Suppression and removal of these pathogenic antibodies with immunosuppressive agents and plasma exchange guide the therapeutic principles of anti-GBM disease (Hudson et al., 2003). Hence, both of these pathogenic antibodies support a definitive role for B lineage cells in human disease.

Immune Complexes, Complement, and Fc Receptors in Disease Pathogenesis

The contributions of antibodies to disease are not solely dependent upon their direct binding to end-organ tissue antigens but also through indirect mechanisms, including IC formation, IC deposition, complement activation, and FcR activation. These complement-activating ICs are found in many autoimmune disorders and deposit in a variety of tissues (e.g., kidney, lung, and blood vessels) to cause end-organ damage (e.g., kidney failure, pulmonary hemorrhage, and vasculitis, respectively). A classical example of an IC-mediated disease is the Hepatitis C-associated syndrome of mixed cryoglobulinemia, in which complement activating IgG and IgM ICs (containing Hepatitis C antigen) are deposited in the skin, kidney, and peripheral nerves to induce cutaneous purpuric lesions, glomerulonephritis, and mononeuritis multiplex, respectively (Trendelenburg and Schifferli, 2003). Treatment is dependent upon the extent of involvement but traditionally geared toward decreasing cryoglobulin production through the use of immunosuppressive therapies and, in severe cases, removal of the pathogenic complexes through plasma exchange.

A role for ICs in autoimmune disorders is also supported by the genetic association of Fc receptor (FcR) polymorphisms and human disease. FcRs for IgG include the high-affinity FcγRI (CD64) receptors and the low-affinity FcγRII (CD32) and FcγRIII (CD16) receptors. Genetic polymorphisms for both low-affinity IgG receptors have been described (Ravetch and Bolland, 2001; Binstadt et al., 2003). The major FcγRIIA polymorphism resides at amino acid 131. FcγRIIA(His131) confers higher affinity for IC binding than FcγRIIA(Arg131). His131/His131 phagocytes are more efficient in IC phagocytosis and clearance than cells bearing the Arg131/Arg131 low-affinity receptor allelic genotype. The major FcγRIIIA polymorphism resides at amino acid 158. Val158 confers high-affinity binding while Phe158 confers low-affinity binding. Patients expressing two copies of FcγRIIA(Arg131/Arg131) or IIIA (Phe158/Phe158) receptors have been reported in some studies but not in others to exhibit increased association with a number of autoimmune diseases, including immune-mediated thrombocytopenia (ITP), SLE, and multiple sclerosis (MS). These polymorphic differences may result not only in prolonged IC circulation, deposition, and tissue damage but may enhance inflammatory and proliferative responses of FcR-bearing cells in disease.

The ability of ICs to activate the complement cascade and liberate proinflammatory complement degradation components that enhance IC clearance, augment phagocyte activation, and modulate cellular and humoral immunity provide additional effector mechanisms by which B cell products can contribute to disease (Holers, 2003; Walport, 2001a, 2001b). In preclinical models, inhibition of complement activation, using soluble forms of complement receptors or antibodies that bind complement degradation products, have demonstrated efficacy in preclinical models of ischemia-induced injury, lupus, and collagen-induced arthritis (Weisman et al., 1990; Couser et al., 1995; Wang et al., 1995, 1996). Translation of some of these principles to the clinics are in progress and will provide us with a greater understanding of these effector pathways in autoimmune disease. A phase II study of the anti-C5 inhibitor antibody eculizumab (5G1.1, Alexion Pharmaceuticals, Cheshire, CT) that prevents the generation of the C5a anaphylatoxin has demonstrated short-term safety and tolerability in patients with rheumatoid arthritis (RA) and clinical benefit compared to control patients (Tesser et al., 2001).

The therapeutic efficacy of IVIG in a limited subset of autoimmune diseases, including ITP and MG, also suggests roles for the contribution of antibodies, complement, and FcRs in B cell-mediated autoimmunity. ITP is a clinical syndrome manifested by immune-mediated destruction of platelets (Beardsley, 2002). These patients demonstrate high levels of autoantibodies directed against platelet surface glycoproteins that are associated with accelerated platelet destruction (McMillan et al., 2001; McMillan, 2003). IVIG is manufactured from pooled human plasma, and its therapeutic effects in ITP involve a multitude of mechanisms that include Fc blockade to decrease clearance of antibody-laden platelets, inhibition of complement deposition, and more

recently evidence for engagement of inhibitory FcγRIIB receptors. In a murine model of autoantibody-induced ITP, the therapeutic effect of IVIG was dependent upon the induced expression of the inhibitory FcγRIIB receptor on splenic macrophages that, in turn, inhibits Fc-mediated opsonization of antibody-bound platelets (Samuelsson et al., 2001). Similarly, the protective effects of IVIG in the K/BxN arthritis model are dependent on the ability of CSF-1-dependent macrophages to upregulate FcγRIIb expression on CSF-1-independent "effector" macrophages (Bruhns et al., 2003). Hence, IVIG may function, in part, by inhibiting effector responses through this inhibitory receptor. Independent of mechanism, the limited therapeutic benefits of IVIG in specific autoimmune disorders support a pathogenic role for antibodies and ICs in these diseases.

The therapeutic application of plasma exchange has provided additional support for the role of antibodies and ICs to implicate B lineage cells in disease (Saydain et al., 2002). Therapeutic apheresis is a technique by which plasma and cellular components are separated and autoantibodies, ICs, or toxins are removed from the plasma. This technique has been modified to include protein A immunoadsorption to specifically extract pathogenic autoantibodies and ICs. While plasma exchange has been used in a wide number of autoimmune diseases, its therapeutic application is accepted as standard of care in only a handful of autoimmune diseases, including anti-GBM disease and MG, and adjunctive therapy in cryoglobulinemia and ITP. Nonetheless, modulation of circulating ICs and pathogenic antibodies by simple removal has proved effective in some of these B lineage-mediated diseases.

B Cell Depletion

The development of therapeutics that specifically target B cells during the past decade has provided an entrée to better translate preclinical observations into human biology and disease. These therapeutics have been led by the use of rituximab (Rituxan®, Genentech, Inc., South San Francisco, CA and Biogen-IDEC, Cambridge, MA; Mabthera®, F.Hoffman-LaRoche, Ltd., Basel, Switzerland), a chimeric monoclonal antibody directed against the CD20 molecule. CD20 is a transmembrane glycoprotein expressed exclusively on normal and malignant B cells. Its expression is regulated during B cell development emerging in late pre-B cells and extinguished on plasma cells. Given the prominent historical role of autoantibodies, the absence of CD20 on plasma cells that presumably are responsible for producing autoantibodies raised fundamental skepticism in applying this therapy to autoimmune diseases. Yet, the efficacious use of Rituxan in a number of autoimmune disorders has brought into question some of the preconceptions of the biology of B lineage cells in autoimmune disorders. Ideally, one would design a B cell-depleting therapy for autoimmunity to meet several criteria: (1) deplete all or most pathogenic B lineage cells or their precursors (assuming that pathogenic cells and their products have a reasonably short lifespan), (2) pathogenic B cell repopulation should not occur or should be significantly delayed upon repletion from early bone marrow progenitors, (3) interrupt or influence non-B cell effector pathogenic pathways that are dependent or downstream of B cell-initiated processes. To date, clinical results exist to support the first two criteria while the third awaits more formal scientific validation.

Clinical data for Rituxan in autoimmune diseases is emerging from a variety of double-blinded placebo-controlled trials though a large amount of data has already emerged from smaller Phase I/II studies and case series reports (Edwards et al., 2002; Silverman and Weisman, 2003). The use of Rituxan in ITP led its entrée into the realm of autoimmune diseases. In four case series reports, 33%–54% of ITP patients, who had failed standard of care corticosteroid and splenectomy therapies, experienced partial or complete responses to Rituxan, in most cases in the absence of concomitant corticosteroids (Saleh et al., 2000; Stasi et al., 2001; Zaja et al., 2003; Cooper et al., 2004). Interestingly, two patterns of clinical responses emerged. Early responders experienced immediate increases in platelet counts after the first or second antibody infusion that were followed by a continued rise until a peak count was achieved between weeks 6 and 10. In contrast, late responders had minimal immediate effects. Rather, they had a delayed effect in which peak counts were achieved within the 6 to 8 week period. These distinguishing patterns suggest that Rituxan may operate through at least two distinct mechanisms in ITP. In the first, the effects of Rituxan are too rapid to be accounted for by depletion of circulating antiplatelet antibodies but potentially could be expanded through Fc receptor-mediated functions. In the latter phase, the sustained effects may function through alterations in anti-platelet antibodies and/or interruption of T-B cell cooperation or other B cell-dependent events in disease pathogenesis. To date, these small clinical studies reveal no direct correlation of anti-platelet antibody levels with clinical responses and raise the likelihood of additional mechanisms by which B lineage cell depletion modulates ITP (see below).

The use of Rituxan in autoimmune disorders is most advanced in the treatment of patients with rheumatoid arthritis (RA). This disease involves a multicellular inflammatory response involving infiltration of lymphocytes and granulocytes, proliferation of synovial fibroblast and macrophages, and neovascularization of the lining surrounding joints. This proliferative process not only induces swelling, erythema, and pain of multiple joints but progress to destruction and loss of bone density and architecture. The diagnosis of RA is aided typically by the presence of low-affinity IgM rheumatoid factor (RF) autoantibodies directed against the Fc portion of IgG. In a multicenter randomized double blind controlled study of 161 patients, those treated with Rituxan and a short course of corticosteroids in conjunction with methotrexate or cyclophosphamide achieved greater clinical responses than patients treated with corticosteroids and methotrexate alone (Shaw et al., 2003; Edwards et al., 2004). Analysis of serologic changes in a smaller series of 22 RA patients demonstrated a 60% decrease in IgM RF and 75% decrease in anti-cyclic citrullinated peptide (CCP) antibodies in Rituxan responders (Cambridge et al., 2003). Despite the longer half-life of IgG, when compared to IgM, IgG RF decreased more rapidly and to a greater extent (80%) than IgM RFs (60%) following Rituxan therapy. In contrast, no

change in anti-pneumococcal capsular polysaccharide IgG antibodies and a 23% decrease in anti-tetanus toxoid IgG antibodies were detected 3 months following treatment. Similar serologic effects have been observed in two additional studies with Rituxan treatment (De Vita et al., 2002; Tuscano, 2002). This preferential decrease in autoantibodies suggests that autoreactive B cells and their corresponding plasma cells may have a shorter lifespan while non-self-reactive plasma cells are relatively more resistant to Rituxan and have a longer lifespan.

Since RF is not thought to play a direct pathogenic role in rheumatoid arthritis, as transfer of RF-positive serum in the *skg* model of RA is not sufficient to confer disease (Sakaguchi et al., 2003), the therapeutic efficacy of Rituxan reported in these studies identifies B lineage cells as playing a key, but undefined, pathogenic role in RA. B cells exist as lymphoid aggregates within the synovium of RA patients and may function as APCs to provide important costimulatory signals in promoting effector T cell expansion (Weyand and Goronzy, 2003). Addition of antibodies that block T and B cell cooperation results in loss of these T-B cell aggregates (Takemura et al., 2001). Hence, B cell depletion may remove a critical component of T-B cell cooperation required for disease. While RF alone is not sufficient to induce disease, RF-containing ICs may promote synoviocytes to induce local release of inflammatory factors and exacerbate the inflammatory cascade. Conversely, decreases in RF and other autoantibodies may attenuate these local inflammatory ICs and result in clinical improvement. Additional clinical studies of synovial B cells prior to and following Ritxuan therapy will be revealing in dissecting the specific mechanistic roles of systemic and local (e.g., synovial) B cells in RA and the effects of Rituxan on RA pathogenesis.

Longer term follow-up analysis of a handful of Rituxan-treated RA patients have been reported and reveal that most undergo clinical relapse. In the study reported by Cambridge and colleagues, 13 of 15 responders relapsed within 17 months following B cell repletion. In all but one patient, return of autoantibodies preceded relapse. Return of autoreactive antibodies, through their corresponding plasma cells and precursors, may occur through a stochastic recapitulation of pathogenic events involving a new set of naive B cells freshly generated from the bone marrow. Supporting this scenario are data on subset composition for repopulating blood cells as well as their VH gene repertoire assayed by single cell PCR (Rouziere et al., 2003). Peripheral B cells from patients with moderate to severe RA have a large component of isotype-switched memory cells with heavily somatically mutated VH genes. During and immediately following B cell repletion, the repertoire is dominated by phenotypically naive cells with germline VH regions. Preceding reemergence of autoantibodies and clinical relapse, the peripheral B cell repertoire begins reaccumulating mutations and slowly converges to resemble the predepletion repertoire. Together, these early studies suggest that pathogenic B cell clones and their progeny can be modulated by Rituxan therapy and that autoreactive and allogeneic responses likely have distinct kinetics of longevity and biology.

Recent studies of four patients with circulatory inhibitors against Factor VIII, an important component of the coagulation cascade, also demonstrate differences between self and non-self antibodies. These patients have circulating FVIII inhibitors (anti-FVIII autoantibodies), despite immunosuppression with corticosteroids and chemotherapeutic agents, which functionally compromise their coagulation cascade, that result in bleeding complications requiring RBC transfusions. Treatment with Rituxan resulted in a rapid decrease in their acquired FVIII inhibitor and return of their endogenous FVIII levels (Wiestner et al., 2002). Interestingly, one of these four patients had mild hemophilia A and received recombinant human FVIII (rFVIII) perioperatively, which appears to have triggered an increase in FVIII inhibitor activity and further compromised his already low FVIIIc level. Administration of Rituxan in this patient resulted in rapid resolution of the autoantibody (against endogenous FVIII) whereas the alloantibody (anti-rFVIII) response persisted for months. Hence, similar to the fate of serum RF, the biologic effects of these autoreactive B cells appear to be far shorter lived than non-self-reactive B cells.

In addition to ITP, RA, and FVIII deficiency, therapeutic effects of Rituxan have been reported in small case series of patients with pemfigus vulgaris (anti-desmoglein-3 antibodies) and pemfigus foliaceus (anti-desmoglein-1 antibodies) (Salopek et al., 2002; Goebeler et al., 2003; Dupuy et al., 2004). In addition to clinical benefit, pathogenic antibody titers decrease, but do not disappear, following Rituxan administration and return at relapse. In at least one patient, clinical relapse and autoantibody increase was documented in the complete absence of peripheral B cells, suggesting a complex relationship between B cells, autoantibodies, and clinical effect. Patients with MG have similarly demonstrated decreases in anti-AChR antibodies, which is accompanied by a slow clinical improvement (Wylam et al., 2003). Finally, patients with IgM-associated polyneuropathies with anti-MAG (myelin-associated glycoprotein) autoantibodies have also been reported to have decreased anti-MAG titers and clinical improvement following Rituxan administration (Levine and Pestronk, 1999; Pestronk et al., 2003). In contrast, two patients with anti-ganglioside IgG antibodies did not improve even up to 1 year following depletion (Rojas-Garcia et al., 2003). This dichotomy of responsiveness does not simply reflect differences between IgM and IgG autoantibodies since other IgG autoantibodies are modulated by Rituxan therapy.

The effects of Rituxan on autoreactive and non-self-reactive antibodies have only recently been studied in SLE patients. Three open label studies have been reported (Leandro et al., 2002; Anolik et al., 2003; Eisenberg, 2003). While the overall efficacy is unknown at this time, the immunologic parameters in these studies demonstrate a further disconnect between B cell depletion and reduction of anti-dsDNA antibodies. Interestingly, these patients have a large percentage of circulating plasmablasts ($CD27^{++}CD20^{-}$), memory ($CD27^{+}CD20^{+}$), and/or germinal center ($CD38^{+}CD20^{+}$) B cells. Analysis of the effects of Rituxan on the survival of these B cell subsets will likely provide greater insights into the half-life and survival factors that regulate these germinal center (GC)-derived cells.

B cell differentiation into Ig-secreting plasma cells has been elegantly explored in humans through the investigation of the CD40/CD40L axis (Arpin et al., 1995). GCs represents a major site to permit crosstalk of antigen-activated T, B, and follicular dendritic cells to promote T and B cell expansion and effector functions. In particular, the CD40/CD40L axis is critical within these GC processes for appropriate generation of memory B cells and long-lived $CD38^{++}CD27^{++}$ antibody-forming cells (plasmacytes). Blockade of the CD40/CD40L axis inhibits activation-induced cytidine deaminase-mediated somatic hypermutation, Ig class switch, GC formation, and maintenance and the generation of long-term B cell memory. Patients with inherited mutations of CD40L are unable to undergo Ig class switch and develop elevated levels of IgM but reduced levels of serum IgG and IgA.

Selective patients with active SLE have significantly elevated numbers of circulating $CD38^{+}IgD^{+}$ preswitch GC cells, $CD38^{+}IgD^{-}$ postswitch GC cells, $CD38^{-}CD27^{+}$ memory cells, and $CD38^{++}$ plasma cells. Some of these cells have variable levels of inappropriate CD40L expression and in vitro can undergo spontaneous proliferation and Ig secretion that is blocked with an anti-CD40L mAb (Grammer et al., 2003; Huang et al., 2002). Treatment of SLE patients with an anti-CD40L antibody (BG9588, 5c8, Biogen-IDEC, Inc.) resulted in substantial decreases in anti-ds DNA antibody, proteinuria, and improved clinical SLEDAI score (a conglomerate measure of clinical and laboratory parameters relevant to the severity of SLE disease). Correspondingly, decreases in $CD38^{+}IgD^{+}$ preswitch GC cells and $CD38^{++}$ plasmacytes with less dramatic effects on $CD38^{+}IgD^{-}$ postswitch GC cells were observed in treated patients. Hence, CD40/CD40L blockade may operate at multiple levels, including inhibiting GC initiation, differentiation of GC cells to $CD38^{++}$ plasmacytes, and interfere with GC maintenance in autoantibody generation.

Together, the body of emerging clinical data involving B cell depletion and modulation in a number of human diseases all indicate a clear pathogenic role for B lineage cells, effector functions, and products in disease pathogenesis. Independent of the direct and indirect roles for antibodies in disease, these data also demonstrate that self-reactive B cells and their products likely exhibit distinct properties as compared to non-self-reactive B cells. Additionally, studies of the CD40/CD40L axis further indicate that targeting of specific subsets of downstream B cell effector function is feasible and will be of benefit in certain human autoimmune disorders.

Mechanisms of B Cell Pathogenicity—Humans and Mice

In this final section, we will link observations on B cell effector functions in human disease with those in murine models of disease and discuss how these parallels might aid in understanding the mechanisms of action for B cell depleting therapies in human autoimmunity. We will begin by continuing the theme of potential differences in the precursors of self- and non-self-reactive antibodies, then discuss non-antibody-dependent contribution of B cells as antigen-presenting cells, and finally, review the role of B cells in lymphogenesis and ectopic neolymphogenesis in disease pathogenesis.

Plasma Cells

B cell pathogenesis in autoimmune diseases has been historically attributed to autoantibodies that, in either soluble or IC forms, are thought to initiate local inflammatory cascades. The plasma cell source of these autoantibodies is much less well characterized in humans with respect to exact phenotype, location, or longevity (Arce et al., 2002; Hauser et al., 2003). Populations of long-lived plasma cells have been well characterized in response against foreign antigens (Manz et al., 1997; Traggiai et al., 2003). These cells, in both mice and humans, are enriched in the bone marrow environment. The existence of long-lived autoimmune plasma cells has been demonstrated in murine models of SLE and suggested in humans. Analysis of these cells in humans, however, has proven difficult due to the extreme low frequency and poor understanding of their precursors and intermediates. Although long- and short-lived terminally differentiated plasma cells downregulate surface expression of CD20, and hence would not be depleted by anti-CD20 therapies, very little is known about their immediate precursors in autoimmune diseases. The minimal decreases observed in total serum Ig and more drastic reductions in autoantibody levels following Rituxan treatment suggest either different precursor sensitivities to depletion or, alternatively, that autoantibody-producing plasma cells are enriched in shorter-lived subsets.

Regardless of whether autoantibodies originate from long- or short-lived precursors, it is widely accepted that most pathogenic autoantibodies increase their affinity through somatic mutations. For normal immune responses, this process occurs within the specialized GC environment. Recent data from a murine model of RF autoantibody generation, however, suggests that there are situations in which high-rate somatic mutations can be generated outside of the GC (William et al., 2002). Microdissection of dividing cell foci demonstrate that mutations are readily detected at the T cell zone—red pulp interface in immunoglobulin transgenic MLR/lpr mice, where the normal Fas/FasL mechanisms for maintaining tolerance are compromised. Paralleling these observations, patients with Fas/FasL pathway defects (human autoimmune lymphoproliferative syndrome), with defects in B and T cell apoptosis, develop in both cellular and humoral autoimmunity.

The exact cellular source of autoantibodies in autoimmune diseases becomes important when one tries to explain the large variability seen in autoantibody reduction in patients. Treatment of nonhuman primates with clinically relevant doses of anti-CD20 antibodies demonstrates significantly different rates for depletion of circulating and lymphoid-laden B cells (Reff et al., 1994; Schroder et al., 2003). Additionally, there appears to be differential sensitivities to depletion of various B cell subsets (Vugmeyster et al., 2003). It is conceivable that the variable effects on autoantibodies may result from the differential depletion of these self- and non-self-reactive plasma cell precursors.

Antibody-Independent Effector Functions

The antibody-independent contributions of B cells in human autoimmunity have also been paralleled in mu-

rine models of disease. The ability of B cells to alter T cell activation and expansion was suggested by the generation of an IgH Tg NZB/W SLE-prone mouse strain expressing a germline immunoglobulin (Ig) transgene (Wellmann et al., 2001). Despite a restricted B cell repertoire, these Ig transgenic mice developed high titers of anti-dsDNA autoantibodies but not IC deposits or glomerulonephritis. They also had improved mortality, when compared to their NZB/W littermates, and displayed reduced T cell activation. A similar finding of B cell-mediated disease has also been demonstrated in the New Zealand obese (NZO) diabetes-prone mouse (Haskell et al., 2002). Hence, autoimmune B cells appear capable of driving T cells to mediate certain subsets of autoimmune manifestations. These distinct contributions are consistent with the identification of genetic elements in the NZM2140 SLE-prone mouse model in which clinical and serologic manifestations of disease can be linked, in part, to T (the *sle3* locus), B (*sle2*), and B-T components (*sle1*) (Nguyen et al., 2002).

The contributions of antibody-independent mechanisms to B cell function is further illustrated in MLR/lpr mice made deficient in their ability to secrete Ig but with intact membrane Ig B cells and B cell effector functions—mIgM MRL/lpr (Chan et al., 1999). Despite the absence of secreted Ig and anti-ds DNA antibodies, these mice still develop interstitial nephritis, vasculitis, and less, but still significant, glomerulonephritis and mortality when compared with secretory sufficient MLR/lpr. In contrast, JHD MLR/lpr mice rendered completely B cell-deficient exhibit minimal disease and mortality (Shlomchik et al., 1994). Hence, effector B cell functions, independent of secreted antibody, are also of paramount importance in disease development. A favored hypothesis to explain these antibody-independent effects is the ability of B cells to present antigen to T cells as part of the pathogenic process. Such a possibility clearly exists, though it should be pointed out that these experimental systems utilize immunoglobulin heavy chain transgenes that can limit the autoreactive B cell receptor repertoire.

Direct inhibition of B/T collaboration, through CD40/CD40L blockade, has been demonstrated to be beneficial in several mouse models of lupus. Anti-CD40L treatment blocks both Ig class switching and somatic mutation, reduces anti-dsDNA IgG antibodies, and induces a variable period of general B and T cell unresponsiveness (Kalled et al., 2001; Wang et al., 2003). In addition, CD40 is required for the development of CD8 memory T cell responses (Bourgeois et al., 2002). Together, these data demonstrate multiple mechanisms by which B/T interaction, presumably through antigen presentation or various cosignals and cytokines, may promote T cell effector functions in disease pathogenesis. Additionally, in MLR/lpr mice with B cells lacking β2-microglobulin, and hence MHC class I expression, the contribution of B cells to CD8 T cell activation can also occur independent of antigen crosspresentation (self-antigen presented on MHC class I to CD8 T cells) (Chan and Shlomchik, 2000). Together, these data open the possibility of APC-dependent and APC-independent mechanisms for B cells in autoimmune mouse models of lupus and potentially in human disease.

Lymphogenesis and Neolymphogenesis

The newly recognized role of B cells in lymphogenesis and ectopic neolymphogenesis may also contribute to autoimmunity and serve as a therapeutic mechanism by which B cell depletion or modulation improves human autoimmunity. Dissection of attraction, migration, and retention signals have demonstrated that lymphoid organs and structures are highly dependent on networks of TNF family molecules, integrins, and chemokines (Fu et al., 1997; Cyster, 1999; Ansel and Cyster, 2001). In generating appropriate lymphoid architecture, B cells play an early requisite role through their interactions with organ stroma and provide a milieu permissive for secondary seeding by T cells and dendritic cells. This sequence of events for neolymphogenesis was elegantly demonstrated by the ectopic introduction of a B cell chemoattractant in the pancreas that initiates a cascade of cellular and developmental events leading to the creation of a new lymphoid structure (Luther et al., 2000). A similar dependence on B cells has also been demonstrated in splenic organogenesis as well as normal M cell development in the gut (Ngo et al., 2001; Golovkina et al., 1999). The complementary scenario has been attempted by pancreatic expression of a T cell chemoattractant. However, in this parallel experimental system, lymphoid organogenesis did not progress to the same stage as with B cells, demonstrating a cellular hierarchy in lymphogenesis (Luther et al., 2002).

B cells appear not only important for the formation but also maintenance of these structures. These observations on lymphogenesis formation and maintenance may be important to the recent description of lymphoid-like follicles in joints and inflamed glands in patients with RA and Sjogren's syndrome, respectively (Weyand and Goronzy, 2003; Groom et al., 2002). Hence, B cell depletion or CD40/CD40L blockade could lead to dissolution of these organized pathogenic lymphoid infiltrates and interfere with maintenance of long-lasting T helper memory as well as dendritic cell localization and function (Moulin et al., 2000; Ngo et al., 2001). Looking at the B cell effector mechanisms in total, it is possible that interrupting all or a combination of the above-described pathways is required for clinical efficacy. Further dissection of these mechanisms in ongoing clinical trials as well as complex models will be required to fully appreciate the multiple B cell functions in immunity and disease.

Future of B Cell Therapies

A final point of discussion for human clinical efficacy in autoimmune diseases is the potential lack of complete anti-CD20 B cell depletion in secondary lymphoid organs. Current protocols of Rituxan administration result in an excess of drug that should efficiently deplete all $CD20^+$ B cells. Explanations for the lack of complete depletion, as has been observed in nonhuman primate studies, could be diverse and span from intrinsic B cell resistance as well as factors derived from the local microenvironment (Reff et al., 1994; Schroder et al., 2003). While intrinsic B cell resistance has been more difficult to address and manipulate in humans, the local B cell microenvironment with the multitude of cells and survival signals is of prime importance for additional clinical intervention. It is conceivable that additional costimula-

tory signals, such as CD40/CD40L, with effects on B cell differentiation and survival could rescue them from depletion. Such survival signals within these microenvironments would not only protect B cells from death but could provide additional growth signals for the expansion of autoimmune B cells. In addition to CD40/CD40L, blocking additional survival signals, such as BLys/BAFF and Toll-like receptors (TLR), would likely be beneficial for targeting downstream effector functions, particularly for autoimmune diseases in which these signals are deregulated.

Patients with SLE and Sjogren's syndrome have elevated levels of serum BLys/BAFF (Zhang et al., 2001; Cheema et al., 2001; Groom et al., 2002; Stohl et al., 2003; Mariette et al., 2003). As BLys/BAFF induces B cell proliferation in vitro and in vivo, blockade of this pathway results in loss of follicular and marginal zone B cells (Moore et al., 1999; Thompson et al., 2000). Moreover, mice overexpressing BLys/BAFF develop autoantibodies, Sjogren's syndrome, and SLE-like autoimmunity (Gross et al., 2000; Khare et al., 2000; Yan et al., 2000). In turn, blockade of this survival signal in murine models of SLE results in significant delay in autoantibody development, decreased proteinuria, and improved mortality (Yan et al., 2001; Gross et al., 2001). Analysis of immunologic parameters in SLE and RA patients presently treated with BLys/BAFF blockade will provide additional insights into human B cell functions (Baker et al., 2003). Should BLys/BAFF prove pivotal in human autoimmune B cell survival in vivo, combination therapies with anti-CD40L, anti-CD20, and BLys/BAFF blockade may provide improved efficacy by modulating distinct effector mechanisms.

TLR ligands, particularly for TLR9 (CpG oligonucleotides, hypomethylated bacterial, and mammalian DNA), are potent activators of dendritic cells but function as cosignals for long-term serologic memory B cells and self-reactive transgenic B cells (Bernasconi et al., 2002; Leadbetter et al., 2002; Viglianti et al., 2003). In addition, activation of TLR9 in vivo accelerates nephritis in the MLR/lpr lupus mouse model and exacerbates experimental allergic encephalitis (Ichikawa et al., 2002; Anders et al., 2004). Consistent with the notion that bacterial or viral infections may act as triggers for clinical flares of autoimmune disorders, triggering of TLR4 (LPS) in mice prone to the development of myocarditis induces clinical autoimmune myocarditis (Eriksson et al., 2003). Hence, activation of TLRs in dendritic cells and B cells may contribute in disease pathogenesis. Interestingly, ongoing clinical studies are utlizing CPG and CPG-conjugated allergens to rebalance a deregulated autoimmune system in the treatment of asthma and other autoimmune disorders (Krieg, 2003). Whether TLRs are differentially utilized among immune and autoimmune B cells is unclear at this time. These ongoing clinical studies combined with preclinical insights in TLR biology should inform us whether this axis can be harnessed in the treatment of autoimmunity.

The beginning of this first decade of the 21st century has been an exciting time with the emergence of a multitude of therapeutic probes to understand human B cell biology. These studies have not only reinforced the importance of autoantibodies and ICs in disease pathogenesis but also provide additional insights into the biology of self-reactive B lineage cells. The second half of this first decade promises to be even more exciting as the impact of these emerging B cell-directed therapies in disease and their immunologic consequences will likely not only improve patient survival and quality of life but will undoubtedly illuminate the multitude of B cell functions in human immunity and autoimmunity.

Acknowledgments

The authors thank Paul Brunetta, Joanne Quan, Lea Sewell, and John Atkinson for critical reading of this manuscript; and Mercedesz Balazs for help with the graphics. Flavius Martin and Andrew Chan are employees of Genentech, Inc.

References

Anasetti, C., Martin, P.J., Storb, R., Appelbaum, F.R., Beatty, P.G., Davis, J., Doney, K., Hill, H.F., Stewart, P., and Sullivan, K.M. (1992). Treatment of acute graft-versus-host disease with a nonmitogenic anti-CD3 monoclonal antibody. Transplantation *54*, 844–851.

Anders, H.J., Vielhauer, V., Eis, V., Linde, Y., Kretzler, M., Perez, D.L., Strutz, F., Bauer, S., Rutz, M., Wagner, H., et al. (2004). Activation of toll-like receptor-9 induces progression of renal disease in MRL-Fas(lpr) mice. FASEB J. *18*, 534–536.

Anolik, J.H., Campbell, D., Felgar, R.E., Young, F., Sanz, I., Rosenblatt, J., and Looney, R.J. (2003). The relationship of FcgammaRIIIa genotype to degree of B cell depletion by rituximab in the treatment of systemic lupus erythematosus. Arthritis Rheum. *48*, 455–459.

Ansel, K.M., and Cyster, J.G. (2001). Chemokines in lymphopoiesis and lymphoid organ development. Curr. Opin. Immunol. *13*, 172–179.

Arce, S., Cassese, G., Hauser, A., Dorner, T., Odendahl, M., Manz, R., Radbruch, A., and Hiepe, F. (2002). The role of long-lived plasma cells in autoimmunity. Immunobiology *206*, 558–562.

Arpin, C., Dechanet, J., Van, K.C., Merville, P., Grouard, G., Briere, F., Banchereau, J., and Liu, Y.J. (1995). Generation of memory B cells and plasma cells in vitro. Science *268*, 720–722.

Baker, K.P., Edwards, B.M., Main, S.H., Choi, G.H., Wager, R.E., Halpern, W.G., Lappin, P.B., Riccobene, T., Abramian, D., Sekut, L., et al. (2003). Generation and characterization of LymphoStat-B, a human monoclonal antibody that antagonizes the bioactivities of B lymphocyte stimulator. Arthritis Rheum. *48*, 3253–3265.

Beardsley, D.S. (2002). Pathophysiology of immune thrombocytopenic purpura. Blood Rev. *16*, 13–14.

Bernasconi, N.L., Traggiai, E., and Lanzavecchia, A. (2002). Maintenance of serological memory by polyclonal activation of human memory B cells. Science *298*, 2199–2202.

Binstadt, B.A., Geha, R.S., and Bonilla, F.A. (2003). IgG Fc receptor polymorphisms in human disease: implications for intravenous immunoglobulin therapy. J. Allergy Clin. Immunol. *111*, 697–703.

Bourgeois, C., Rocha, B., and Tanchot, C. (2002). A role for CD40 expression on CD8+ T cells in the generation of CD8+ T cell memory. Science *297*, 2060–2063.

Brucato, A., Cimaz, R., and Stramba-Badiale, M. (2002). Neonatal lupus. Clin. Rev. Allergy Immunol. *23*, 279–299.

Bruhns, P., Samuelsson, A., Pollard, J.W., and Ravetch, J.V. (2003). Colony-stimulating factor-1-dependent macrophages are responsible for IVIG protection in antibody-induced autoimmune disease. Immunity *18*, 573–581.

Buyon, J.P., and Clancy, R.M. (2003). Neonatal lupus: review of proposed pathogenesis and clinical data from the US-based Research Registry for Neonatal Lupus. Autoimmunity *36*, 41–50.

Cambridge, G., Leandro, M.J., Edwards, J.C., Ehrenstein, M.R., Salden, M., Bodman-Smith, M., and Webster, A.D. (2003). Serologic changes following B lymphocyte depletion therapy for rheumatoid arthritis. Arthritis Rheum. *48*, 2146–2154.

Cersosimo, R.J. (2003). Monoclonal antibodies in the treatment of cancer, part 2. Am. J. Health Syst. Pharm. *60*, 1631–1641.

Chan, O.T., and Shlomchik, M.J. (2000). Cutting edge: B cells promote CD8+ T cell activation in MRL-Fas(lpr) mice independently of MHC class I antigen presentation. J. Immunol. *164*, 1658–1662.

Chan, O.T., Hannum, L.G., Haberman, A.M., Madaio, M.P., and Shlomchik, M.J. (1999). A novel mouse with B cells but lacking serum antibody reveals an antibody-independent role for B cells in murine lupus. J. Exp. Med. *189*, 1639–1648.

Cheema, G.S., Roschke, V., Hilbert, D.M., and Stohl, W. (2001). Elevated serum B lymphocyte stimulator levels in patients with systemic immune-based rheumatic diseases. Arthritis Rheum. *44*, 1313–1319.

Cohen, S., Hurd, E., Cush, J., Schiff, M., Weinblatt, M.E., Moreland, L.W., Kremer, J., Bear, M.B., Rich, W.J., and McCabe, D. (2002). Treatment of rheumatoid arthritis with anakinra, a recombinant human interleukin-1 receptor antagonist, in combination with methotrexate: results of a twenty-four-week, multicenter, randomized, double-blind, placebo-controlled trial. Arthritis Rheum. *46*, 614–624.

Cooper, M.D., Peterson, R.D., and Good, R.A. (1965). Delineation of the thymic and bursal lymphoid system in the chicken. Nature *205*, 143–146.

Cooper, M.D., Kearney, J.F., Gathings, W.E., and Lawton, A.R. (1980). Effects of anti-Ig antibodies on the development and differentiation of B cells. Immunol. Rev. *52*, 29–53.

Cooper, N., Stasi, R., Cunningham-Rundles, S., Feuerstein, M.A., Leonard, J.P., Amadori, S., and Bussel, J.B. (2004). The efficacy and safety of B-cell depletion with anti-CD20 monoclonal antibody in adults with chronic immune thrombocytopenic purpura. Br. J. Haematol. *125*, 232–239.

Couser, W.G., Johnson, R.J., Young, B.A., Yeh, C.G., Toth, C.A., and Rudolph, A.R. (1995). The effects of soluble recombinant complement receptor 1 on complement-mediated experimental glomerulonephritis. J. Am. Soc. Nephrol. *5*, 1888–1894.

Cyster, J.G. (1999). Chemokines and cell migration in secondary lymphoid organs. Science *286*, 2098–2102.

Davidson, A., and Diamond, B. (2001). Autoimmune diseases. N. Engl. J. Med. *345*, 340–350.

De Vita, S., Zaja, F., Sacco, S., De Candia, A., Fanin, R., and Ferraccioli, G. (2002). Efficacy of selective B cell blockade in the treatment of rheumatoid arthritis: evidence for a pathogenetic role of B cells. Arthritis Rheum. *46*, 2029–2033.

Dupuy, A., Viguier, M., Bedane, C., Cordoliani, F., Blaise, S., Aucouturier, F., Bonnetblanc, J.M., Morel, P., Dubertret, L., and Bachelez, H. (2004). Treatment of refractory pemphigus vulgaris with rituximab (anti-CD20 monoclonal antibody). Arch. Dermatol. *140*, 91–96.

Edwards, J.C., Leandro, M.J., and Cambridge, G. (2002). B-lymphocyte depletion therapy in rheumatoid arthritis and other autoimmune disorders. Biochem. Soc. Trans. *30*, 824–828.

Edwards, J.C., Szczepanski, L., Szechinski, J., Filipowicz-Sosnowska, A., Close, D.R., Stevens, R.M., and Shaw, T. (2004). Efficacy of the novel B cell targeted therapy, Rituximab, in patients with active rheumatoid arthritis. N. Engl. J. Med., in press.

Eisenberg, R. (2003). Mechanisms of autoimmunity. Immunol. Res. *27*, 203–218.

Eriksson, U., Ricci, R., Hunziker, L., Kurrer, M.O., Oudit, G.Y., Watts, T.H., Sonderegger, I., Bachmaier, K., Kopf, M., and Penninger, J.M. (2003). Dendritic cell-induced autoimmune heart failure requires cooperation between adaptive and innate immunity. Nat. Med. *9*, 1484–1490.

Fu, Y.X., Huang, G., Matsumoto, M., Molina, H., and Chaplin, D.D. (1997). Independent signals regulate development of primary and secondary follicle structure in spleen and mesenteric lymph node. Proc. Natl. Acad. Sci. USA *94*, 5739–5743.

Goebeler, M., Herzog, S., Brocker, E.B., and Zillikens, D. (2003). Rapid response of treatment-resistant pemphigus foliaceus to the anti-CD20 antibody rituximab. Br. J. Dermatol. *149*, 899–901.

Golovkina, T.V., Shlomchik, M., Hannum, L., and Chervonsky, A. (1999). Organogenic role of B lymphocytes in mucosal immunity. Science *286*, 1965–1968.

Gottlieb, A.B. (2003). Clinical research helps elucidate the role of tumor necrosis factor-alpha in the pathogenesis of T1-mediated immune disorders: use of targeted immunotherapeutics as pathogenic probes. Lupus *12*, 190–194.

Grammer, A.C., Slota, R., Fischer, R., Gur, H., Girschick, H., Yarboro, C., Illei, G.G., and Lipsky, P.E. (2003). Abnormal germinal center reactions in systemic lupus erythematosus demonstrated by blockade of CD154-CD40 interactions. J. Clin. Invest. *112*, 1506–1520.

Groom, J., Kalled, S.L., Cutler, A.H., Olson, C., Woodcock, S.A., Schneider, P., Tschopp, J., Cachero, T.G., Batten, M., Wheway, J., et al. (2002). Association of BAFF/BLyS overexpression and altered B cell differentiation with Sjogren's syndrome. J. Clin. Invest. *109*, 59–68.

Gross, J.A., Johnston, J., Mudri, S., Enselman, R., Dillon, S.R., Madden, K., Xu, W., Parrish-Novak, J., Foster, D., Lofton-Day, C., et al. (2000). TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. Nature *404*, 995–999.

Gross, J.A., Dillon, S.R., Mudri, S., Johnston, J., Littau, A., Roque, R., Rixon, M., Schou, O., Foley, K.P., Haugen, H., et al. (2001). TACI-Ig neutralizes molecules critical for B cell development and autoimmune disease. Impaired B cell maturation in mice lacking BLyS. Immunity *15*, 289–302.

Haskell, B.D., Flurkey, K., Duffy, T.M., Sargent, E.E., and Leiter, E.H. (2002). The diabetes-prone NZO/HILt strain. I. Immunophenotypic comparison to the related NZB/BINJ and NZW/LacJ strains. Lab. Invest. *82*, 833–842.

Hauser, A.E., Muehlinghaus, G., Manz, R.A., Cassese, G., Arce, S., Debes, G.F., Hamann, A., Berek, C., Lindenau, S., Doerner, T., et al. (2003). Long-lived plasma cells in immunity and inflammation. Ann. N Y Acad. Sci. *987*, 266–269.

Holers, V.M. (2003). The complement system as a therapeutic target in autoimmunity. Clin. Immunol. *107*, 140–151.

Huang, W., Sinha, J., Newman, J., Reddy, B., Budhai, L., Furie, R., Vaishnaw, A., and Davidson, A. (2002). The effect of anti-CD40 ligand antibody on B cells in human systemic lupus erythematosus. Arthritis Rheum. *46*, 1554–1562.

Hudson, B.G., Tryggvason, K., Sundaramoorthy, M., and Neilson, E.G. (2003). Alport's syndrome, Goodpasture's syndrome, and type IV collagen. N. Engl. J. Med. *348*, 2543–2556.

Ichikawa, H.T., Williams, L.P., and Segal, B.M. (2002). Activation of APCs through CD40 or Toll-like receptor 9 overcomes tolerance and precipitates autoimmune disease. J. Immunol. *169*, 2781–2787.

Juweid, M. (2003). Technology evaluation: epratuzumab, Immunomedics/Amgen. Curr. Opin. Mol. Ther. *5*, 192–198.

Kalled, S.L., Cutler, A.H., and Ferrant, J.L. (2001). Long-term anti-CD154 dosing in nephritic mice is required to maintain survival and inhibit mediators of renal fibrosis. Lupus *10*, 9–22.

Kamradt, T., and Mitchison, N.A. (2001). Tolerance and autoimmunity. N. Engl. J. Med. *344*, 655–664.

Keystone, E.C. (2003). Abandoned therapies and unpublished trials in rheumatoid arthritis. Curr. Opin. Rheumatol. *15*, 253–258.

Keystone, E.C., Haraoui, B., and Bykerk, V.P. (2003). Role of adalimumab in the treatment of early rheumatoid arthritis. Clin. Exp. Rheumatol. *21*, S198–S199.

Khare, S.D., Sarosi, I., Xia, X.Z., McCabe, S., Miner, K., Solovyev, I., Hawkins, N., Kelley, M., Chang, D., Van, G., et al. (2000). Severe B cell hyperplasia and autoimmune disease in TALL-1 transgenic mice. Proc. Natl. Acad. Sci. USA *97*, 3370–3375.

Kremer, J.M., Westhovens, R., Leon, M., Di Giorgio, E., Alten, R., Steinfeld, S., Russell, A., Dougados, M., Emery, P., Nuamah, I.F., et al. (2003). Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. N. Engl. J. Med. *349*, 1907–1915.

Krieg, A.M. (2003). CpG motifs: the active ingredient in bacterial extracts? Nat. Med. *9*, 831–835.

Leadbetter, E.A., Rifkin, I.R., Hohlbaum, A.M., Beaudette, B.C., Shlomchik, M.J., and Marshak-Rothstein, A. (2002). Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. Nature *416*, 603–607.

Leandro, M.J., Edwards, J.C., Cambridge, G., Ehrenstein, M.R., and

Isenberg, D.A. (2002). An open study of B lymphocyte depletion in systemic lupus erythematosus. Arthritis Rheum. *46*, 2673–2677.

Levine, T.D., and Pestronk, A. (1999). IgM antibody-related polyneuropathies: B-cell depletion chemotherapy using Rituximab. Neurology *52*, 1701–1704.

Lipsky, P.E., van der Heijde, D.M., St Clair, E.W., Furst, D.E., Breedveld, F.C., Kalden, J.R., Smolen, J.S., Weisman, M., Emery, P., Feldmann, M., et al. (2000). Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy study group. N. Engl. J. Med. *343*, 1594–1602.

Luther, S.A., Lopez, T., Bai, W., Hanahan, D., and Cyster, J.G. (2000). BLC expression in pancreatic islets causes B cell recruitment and lymphotoxin-dependent lymphoid neogenesis. Immunity *12*, 471–481.

Luther, S.A., Bidgol, A., Hargreaves, D.C., Schmidt, A., Xu, Y., Paniyadi, J., Matloubian, M., and Cyster, J.G. (2002). Differing activities of homeostatic chemokines CCL19, CCL21, and CXCL12 in lymphocyte and dendritic cell recruitment and lymphoid neogenesis. J. Immunol. *169*, 424–433.

Manz, R.A., Thiel, A., and Radbruch, A. (1997). Lifetime of plasma cells in the bone marrow. Nature *388*, 133–134.

Mariette, X., Roux, S., Zhang, J., Bengoufa, D., Lavie, F., Zhou, T., and Kimberly, R. (2003). The level of BLyS (BAFF) correlates with the titre of autoantibodies in human Sjogren's syndrome. Ann. Rheum. Dis. *62*, 168–171.

McCarthy, H., Ottensmeier, C.H., Hamblin, T.J., and Stevenson, F.K. (2003). Anti-idiotype vaccines. Br. J. Haematol. *123*, 770–781.

McMillan, R. (2003). Antiplatelet antibodies in chronic adult immune thrombocytopenic purpura: assays and epitopes. J. Pediatr. Hematol. Oncol. *25*, S57–S61.

McMillan, R., Lopez-Dee, J., and Loftus, J.C. (2001). Autoantibodies to alpha(IIb)beta(3) in patients with chronic immune thrombocytopenic purpura bind primarily to epitopes on alpha(IIb). Blood *97*, 2171–2172.

Moore, P.A., Belvedere, O., Orr, A., Pieri, K., LaFleur, D.W., Feng, P., Soppet, D., Charters, M., Gentz, R., Parmelee, D., et al. (1999). BLyS: member of the tumor necrosis factor family and B lymphocyte stimulator. Science *285*, 260–263.

Moulin, V., Andris, F., Thielemans, K., Maliszewski, C., Urbain, J., and Moser, M. (2000). B lymphocytes regulate dendritic cell (DC) function in vivo: increased interleukin 12 production by DCs from B cell-deficient mice results in T helper cell type 1 deviation. J. Exp. Med. *192*, 475–482.

Ngo, V.N., Cornall, R.J., and Cyster, J.G. (2001). Splenic T zone development is B cell dependent. J. Exp. Med. *194*, 1649–1660.

Nguyen, C., Limaye, N., and Wakeland, E.K. (2002). Susceptibility genes in the pathogenesis of murine lupus. Arthritis Res. *4*, S255–S263.

Pascual, M., Mach-Pascual, S., and Schifferli, J.A. (1997). Paraproteins and complement depletion: pathogenesis and clinical syndromes. Semin. Hematol. *34*, 40–48.

Pestronk, A., Florence, J., Miller, T., Choksi, R., Al Lozi, M.T., and Levine, T.D. (2003). Treatment of IgM antibody associated polyneuropathies using rituximab. J. Neurol. Neurosurg. Psychiatry *74*, 485–489.

Pisetsky, D.S. (2000a). Anti-DNA and autoantibodies. Curr. Opin. Rheumatol. *12*, 364–368.

Pisetsky, D.S. (2000b). Tumor necrosis factor blockers in rheumatoid arthritis. N. Engl. J. Med. *342*, 810–811.

Ravetch, J.V., and Bolland, S. (2001). IgG Fc receptors. Annu. Rev. Immunol. *19*, 275–290.

Reff, M.E., Carner, K., Chambers, K.S., Chinn, P.C., Leonard, J.E., Raab, R., Newman, R.A., Hanna, N., and Anderson, D.R. (1994). Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood *83*, 435–445.

Reichlin, M. (2003). Ribosomal P antibodies and CNS lupus. Lupus *12*, 916–918.

Richman, D.P., and Agius, M.A. (2003). Treatment of autoimmune myasthenia gravis. Neurology *61*, 1652–1661.

Robak, T. (2004). Monoclonal antibodies in the treatment of autoimmune cytopenias. Eur. J. Haematol. *72*, 79–88.

Rojas-Garcia, R., Gallardo, E., de Andres, I., de Luna, N., Juarez, C., Sanchez, P., and Illa, I. (2003). Chronic neuropathy with IgM anti-ganglioside antibodies: lack of long term response to rituximab. Neurology *61*, 1814–1816.

Rouziere, A.S., Kneitz, C., Dorner, T., and Tony, H.P. (2003). B-cell depletion by anti-CD20 antibody treatment in rheumatoid arthritis modulates the B-cell repertoire. Arthritis Rheum. *48*, S138.

Ruiz-Irastorza, G., Khamashta, M.A., and Hughes, G.R. (2002). Hughes syndrome crosses boundaries. Autoimmun. Rev. *1*, 43–48.

Sakaguchi, N., Takahashi, T., Hata, H., Nomura, T., Tagami, T., Yamazaki, S., Sakihama, T., Matsutani, T., Negishi, I., Nakatsuru, S., et al. (2003). Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. Nature *426*, 454–460.

Saleh, M.N., Gutheil, J., Moore, M., Bunch, P.W., Butler, J., Kunkel, L., Grillo-Lopez, A.J., and Lobuglio, A.F. (2000). A pilot study of the anti-CD20 monoclonal antibody rituximab in patients with refractory immune thrombocytopenia. Semin. Oncol. *27*, 99–103.

Salopek, T.G., Logsetty, S., and Tredget, E.E. (2002). Anti-CD20 chimeric monoclonal antibody (rituximab) for the treatment of recalcitrant, life-threatening pemphigus vulgaris with implications in the pathogenesis of the disorder. J. Am. Acad. Dermatol. *47*, 785–788.

Samuelsson, A., Towers, T.L., and Ravetch, J.V. (2001). Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. Science *291*, 484–486.

Saydain, G., George, L., and Raoof, S. (2002). New therapies: plasmapheresis, intravenous immunoglobulin, and monoclonal antibodies. Crit. Care Clin. *18*, 957–975.

Schroder, C., Azimzadeh, A.M., Wu, G., Price, J.O., Atkinson, J.B., and Pierson, R.N. (2003). Anti-CD20 treatment depletes B-cells in blood and lymphatic tissue of cynomolgus monkeys. Transpl. Immunol. *12*, 19–28.

Shanahan, J.C., Moreland, L.W., and Carter, R.H. (2003). Upcoming biologic agents for the treatment of rheumatic diseases. Curr. Opin. Rheumatol. *15*, 226–236.

Shaw, T., Quan, J., and Totoritis, M.C. (2003). B cell therapy for rheumatoid arthritis: the rituximab (anti-CD20) experience. Ann. Rheum. Dis. *62*, ii55–ii59.

Shlomchik, M.J., Madaio, M.P., Ni, D., Trounstein, M., and Huszar, D. (1994). The role of B cells in lpr/lpr-induced autoimmunity. J. Exp. Med. *180*, 1295–1306.

Silberstein, L.E. (1994). B-cell origin of cold agglutinins. Adv. Exp. Med. Biol. *347*, 193–205.

Silverman, G.J., and Weisman, S. (2003). Rituximab therapy and autoimmune disorders: prospects for anti-B cell therapy. Arthritis Rheum. *48*, 1484–1492.

Stasi, R., Pagano, A., Stipa, E., and Amadori, S. (2001). Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idiopathic thrombocytopenic purpura. Blood *98*, 952–957.

Stohl, W., Metyas, S., Tan, S.M., Cheema, G.S., Oamar, B., Xu, D., Roschke, V., Wu, Y., Baker, K.P., and Hilbert, D.M. (2003). B lymphocyte stimulator overexpression in patients with systemic lupus erythematosus: longitudinal observations. Arthritis Rheum. *48*, 3475–3486.

Takemura, S., Klimiuk, P.A., Braun, A., Goronzy, J.J., and Weyand, C.M. (2001). T cell activation in rheumatoid synovium is B cell dependent. J. Immunol. *167*, 4710–4718.

Tesser, J., Kivitz, A., Fleischmann, R., Mojcik, C.F., Bombara, M., and Burch, F. (2001). Safety and efficacy of the humanized anti-C5 antibody h5G1.1 in patients with rheumatoid arthritis. Arthritis Rheum. *44*, S274.

Thompson, J.S., Schneider, P., Kalled, S.L., Wang, L., Lefevre, E.A., Cachero, T.G., MacKay, F., Bixler, S.A., Zafari, M., Liu, Z.Y., et al. (2000). BAFF binds to the tumor necrosis factor receptor-like mole-

cule B cell maturation antigen and is important for maintaining the peripheral B cell population. J. Exp. Med. *192*, 129–135.

Traggiai, E., Puzone, R., and Lanzavecchia, A. (2003). Antigen dependent and independent mechanisms that sustain serum antibody levels. Vaccine *21*, S35–S37.

Trendelenburg, M., and Schifferli, J.A. (2003). Cryoglobulins in chronic hepatitis C virus infection. Clin. Exp. Immunol. *133*, 153–155.

Treon, S.P., and Anderson, K.C. (2000). The use of rituximab in the treatment of malignant and nonmalignant plasma cell disorders. Semin. Oncol. *27*, 79–85.

Tuscano, J.M. (2002). Successful treatment of infliximab-refractory rheumatoid arthritis with rituximab. Arthritis Rheum. *46*, 3420.

Viglianti, G.A., Lau, C.M., Hanley, T.M., Miko, B.A., Shlomchik, M.J., and Marshak-Rothstein, A. (2003). Activation of autoreactive B cells by CpG dsDNA. Immunity *19*, 837–847.

Vincent, A. (2002). Unravelling the pathogenesis of myasthenia gravis. Nat. Rev. Immunol. *2*, 797–804.

Vugmeyster, Y., Howell, K., McKeever, K., Combs, D., and Canova-Davis, E. (2003). Differential in vivo effects of rituximab on two B-cell subsets in cynomolgus monkeys. Int. Immunopharmacol. *3*, 1477–1481.

Walport, M.J. (2001a). Complement. First of two parts. N. Engl. J. Med. *344*, 1058–1066.

Walport, M.J. (2001b). Complement. Second of two parts. N. Engl. J. Med. *344*, 1140–1144.

Wang, Y., Rollins, S.A., Madri, J.A., and Matis, L.A. (1995). Anti-C5 monoclonal antibody therapy prevents collagen-induced arthritis and ameliorates established disease. Proc. Natl. Acad. Sci. USA *92*, 8955–8959.

Wang, Y., Hu, Q., Madri, J.A., Rollins, S.A., Chodera, A., and Matis, L.A. (1996). Amelioration of lupus-like autoimmune disease in NZB/WF1 mice after treatment with a blocking monoclonal antibody specific for complement component C5. Proc. Natl. Acad. Sci. USA *93*, 8563–8568.

Wang, X., Huang, W., Schiffer, L.E., Mihara, M., Akkerman, A., Hiromatsu, K., and Davidson, A. (2003). Effects of anti-CD154 treatment on B cells in murine systemic lupus erythematosus. Arthritis Rheum. *48*, 495–506.

Ward, J.H. (2001). Autoimmunity in chronic lymphocytic leukemia. Curr. Treat. Options Oncol. *2*, 253–257.

Weinblatt, M.E., Kremer, J.M., Bankhurst, A.D., Bulpitt, K.J., Fleischmann, R.M., Fox, R.I., Jackson, C.G., Lange, M., and Burge, D.J. (1999). A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. N. Engl. J. Med. *340*, 253–259.

Weisman, H.F., Bartow, T., Leppo, M.K., Marsh, H.C., Jr., Carson, G.R., Concino, M.F., Boyle, M.P., Roux, K.H., Weisfeldt, M.L., and Fearon, D.T. (1990). Soluble human complement receptor type 1: in vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. Science *249*, 146–151.

Wellmann, U., Letz, M., Schneider, A., Amann, K., and Winkler, T.H. (2001). An Ig mu-heavy chain transgene inhibits systemic lupus erythematosus immunopathology in autoimmune (NZB x NZW) F1 mice. Int. Immunol. *13*, 1461–1469.

Weyand, C.M., and Goronzy, J.J. (2003). Ectopic germinal center formation in rheumatoid synovitis. Ann. N Y Acad. Sci. *987*, 140–149.

Wiestner, A., Cho, H.J., Asch, A.S., Michelis, M.A., Zeller, J.A., Peerschke, E.I., Weksler, B.B., and Schechter, G.P. (2002). Rituximab in the treatment of acquired factor VIII inhibitors. Blood *100*, 3426–3428.

William, J., Euler, C., Christensen, S., and Shlomchik, M.J. (2002). Evolution of autoantibody responses via somatic hypermutation outside of germinal centers. Science *297*, 2066–2070.

Wylam, M.E., Anderson, P.M., Kuntz, N.L., and Rodriguez, V. (2003). Successful treatment of refractory myasthenia gravis using rituximab: a pediatric case report. J. Pediatr. *143*, 674–677.

Yan, M., Marsters, S.A., Grewal, I.S., Wang, H., Ashkenazi, A., and Dixit, V.M. (2000). Identification of a receptor for BLyS demonstrates a crucial role in humoral immunity. Nat. Immunol. *1*, 37–41.

Yan, M., Brady, J.R., Chan, B., Lee, W.P., Hsu, B., Harless, S., Cancro, M., Grewal, I.S., and Dixit, V.M. (2001). Identification of a novel receptor for B lymphocyte stimulator that is mutated in a mouse strain with severe B cell deficiency. Curr. Biol. *11*, 1547–1552.

Zaja, F., Vianelli, N., Sperotto, A., De Vita, S., Iacona, I., Zaccaria, A., Masolini, P., Tomadini, V., Tani, M., Molinari, A.L., et al. (2003). B-cell compartment as the selective target for the treatment of immune thrombocytopenias. Haematologica *88*, 538–546.

Zhang, J., Roschke, V., Baker, K.P., Wang, Z., Alarcon, G.S., Fessler, B.J., Bastian, H., Kimberly, R.P., and Zhou, T. (2001). Cutting edge: a role for B lymphocyte stimulator in systemic lupus erythematosus. J. Immunol. *166*, 6–10.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Molecular Immunology 43 (2006) 107–121
Molecular Immunology
www.elsevier.com/locate/molimm

Review

Strategies of therapeutic complement inhibition

Tom E. Mollnes [a,*], Michael Kirschfink [b]

[a] *Institute of Immunology, Rikshospitalet University Hospital and University of Oslo, N-0027 Oslo, Norway*
[b] *Institute of Immunology, University of Heidelberg, Germany*

Abstract

The involvement of complement in the pathogenesis of a great number of partly life threatening diseases defines the importance to develop inhibitors which specifically interfere with its deleterious action. Endogenous soluble complement-inhibitors, antibodies or low molecular weight antagonists, either blocking key proteins of the cascade reaction or neutralizing the action of the complement-derived anaphylatoxins have successfully been tested in various animal models over the past years. Promising results consequently led to first clinical trials. This review is focused on different approaches for the development of inhibitors, on their site of action in the cascade, on possible indications for complement inhibition based on experimental animal data, and on potential side effects of such treatment.
© 2005 Elsevier Ltd. All rights reserved.

Keywords: Complement; Inflammatory disorders; Inhibitors; Therapy

1. Introduction

Complement as an essential component of the immune system is of substantial relevance for the destruction of invading microorganisms and for maintaining tissue homeostasis including the protection against autoimmune diseases (Walport, 2001a,b). However, excessive or uncontrolled complement activation significantly contributes to undesired tissue damage. Following complement activation, biologically active peptides, such as C5a and C3a elicit a number of proinflammatory effects, including the recruitment of leukocytes, degranulation of phagocytic cells, mast cells and basophils, smooth muscle contraction, and increase of vascular permeability. Upon complement-dependent cell activation, the inflammatory response is further amplified by subsequent generation of toxic oxygen radicals and the induction of synthesis and release of arachidonic acid metabolites and cytokines. Consequently, complement activation presents a considerable risk of harming the host by directly and indirectly mediating inflammatory tissue destruction.

Under physiological conditions, activation of complement is effectively controlled by the coordinated action of soluble as well as membrane-associated regulatory proteins. Soluble complement regulators, such as C1-inhibitor, anaphylotoxin inhibitor (serum carboxypeptidase N), C4b binding protein (C4BP), factors H and I, clusterin and S-protein (vitronectin), restrict the action of complement in body fluids at multiple sites of the cascade reaction. In addition, each individual cell is protected against the attack of homologous complement by surface proteins, such as the complement receptor 1 (CR1, CD35), the membrane cofactor protein (MCP, CD46) as well as by the glycosylphosphatidylinositol (GPI)-anchored proteins, decay-accelerating factor (DAF, CD55) and CD59. When complement is improperly activated, these regulatory mechanisms may be overwhelmed, resulting in tissue destruction and disease.

Clinical and experimental evidence underlines the prominent role of complement in the pathogenesis of numerous inflammatory diseases (Szebeni, 2004). The list of such conditions is rapidly growing, including immune-complex diseases such as rheumatoid arthritis and systemic lupus erythematosus, ischemia–reperfusion (I/R) injury locally manifested as infarctions or systemically as a post-ischemic inflammatory syndrome, systemic inflammatory response

* Corresponding author. Tel.: +47 90630015; fax: +47 23073510.
E-mail address: t.e.mollnes@labmed.uio.no (T.E. Mollnes).

0161-5890/$ – see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.molimm.2005.06.014

syndrome (SIRS) and acute respiratory distress syndrome (ARDS), septic shock, trauma, burns, acid aspiration to the lungs, renal diseases, inflammatory and degenerative diseases in the nervous system, arteriosclerosis, transplant rejection and inflammatory complications seen after cardiopulmonary bypass and haemodialysis. In principle, when inflammation is involved in the pathogenesis, complement has to be considered as a possible mediator in the disease process (Mollnes et al., 2002).

In recent years, great progress has been made in complement analysis to better define disease severity, evolution and response to therapy. Modern diagnostic technologies which focus on the quantification of complement activation products now provide a comprehensive insight into the activation state of the system. Thus, to focus on complement inhibition appears to be a logical approach in order to arrest the process of inflammatory disorders. Due to the vast amount of data already available in the literature, only parts of it can be included here. For further reading, see previous general reviews, e.g. (Lambris and Holers, 2000; Asghar and Pasch, 2000; Kirschfink, 2001; Quigg, 2002; Morgan and Harris, 2003; Mollnes, 2004). In addition, a number of review papers dealing either with a certain candidate inhibitor or treatment of a certain disease have been published the last decade.

2. General aspects of complement inhibition therapy

2.1. Complement in inflammation

The pathophysiology of inflammation is complex and diverse with innumerable mutually interacting mediators (see also preceding reviews in this issue). The role of complement in the inflammatory network may also vary with the different conditions, from being crucial to just epiphenomenonal. An essential issue is whether complement activation, by activating leukocytes, endothelial cells, platelets and other cells, induces secondary inflammatory mediators which may contribute to or even determine tissue damage, such as cytokines, reactive oxygen species, arachidonic acid metabolites and expression of adhesion molecules. On the other hand, some of these mediators may be primarily induced and activate complement as a secondary mechanism of inflammation. The question frequently raised is what comes first, the chicken or the egg. This has significant implication to the rationale and design of anti-inflammatory therapy in general and for complement inhibition in particular. In principle, it would be most effective to block upstream of the inflammation cascade, e.g. the primary inducer(s). The matter is, however, rather complex since some of the inflammatory mediators may have mainly adverse effects on tissue homeostasis whereas others are beneficial. Thus, a careful dissection of the pro and anti-inflammatory effects of complement is needed to identify and inhibit those mediators contributing to the tissue damage and to spare those which are needed for a balanced homeostasis.

2.2. Rationale for complement inhibition in human disease

Targeting complement as a therapeutic goal requires detailed knowledge about the activation mechanisms and mediators responsible for the inflammation, enabling optimal inhibitory treatment for the actual condition. Many mechanisms have been experimentally elucidated, particularly in I/R injury, but the field is still in its infancy. Successful treatment with a specific complement-inhibitor is the ultimate proof that complement plays an essential role in the actual disease. The design of clinical trials has to rely on data from animal disease models. However, due to species differences, results from animal studies are not necessarily applicable to humans. Finally, only their clinical application determines to what extent a particular disease will benefit from such treatment. Only comparable to primary deficiency states as experiments of nature, specific therapeutic inhibition will provide the ultimate concept validation for the role of complement in the pathogenesis of human diseases.

Activation of complement, as detected by increased levels of complement activation products in plasma samples, is known to occur in a number of disease conditions. However, increased complement activation does not necessarily imply that complement is of importance in the pathogenesis. On the other hand, normal systemic levels of activation products is seen in many conditions where local complement activation is likely to play a role in local tissue damage. In such conditions, complement activation can be detected in tissue biopsies or in the local body fluid. In general, an ongoing systemic activation is required for increased activation products to be detected in a plasma sample. The site of activation will determine the inhibitor to be used and its route of application.

2.3. Potential sites in the complement cascade to be inhibited (Fig. 1)

The majority of approaches to inhibit complement has focused on either blocking at the level of C3 implying a general and broad inhibition of the system, or on selective blocking of C5 activation with subsequent inhibition of C5a and C5b-9 (TCC) formation (Fig. 1). In favor of the former is the argument that if complement activation is detrimental, it should be reasonable to extend the system's inhibition as wide and complete as possible. On the other hand, blocking of the terminal pathway allows C3 activation as required to mount an effective defense against foreign invaders. In fact, both arguments over-simplify the situation and have limited clinical validation. The mechanisms of complement activation and – even more – the contribution of this activation to the pathophysiology of different clinical conditions are so diverse that a differential approximation to this issue is required and several strategies must be considered.

One such strategy will be to target the initiation step of complement activation, provided detailed knowledge on the activation mechanism. Each of the three initial activation

Samsung Bioepis Exhibit No. 1002
Page 945

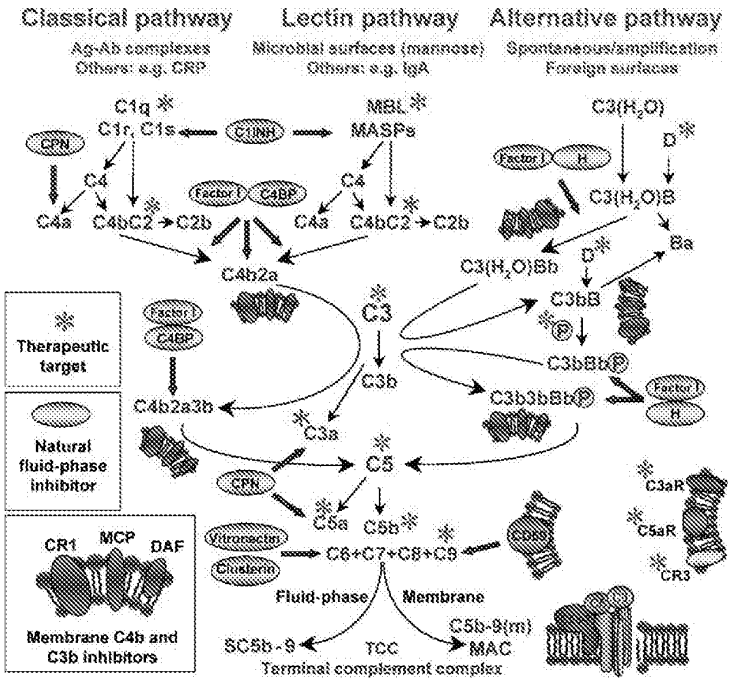


Fig. 1. Complement cascade reaction and physiological regulation. Various reagents with potential therapeutic applications have been developed to target complement activation and function (potential targets are indicated by red asterisks). For details, see Mollnes et al. (2002), where the figure was originally published (reprinted here with permission from Elsevier).

pathways can be blocked separately. For example, inhibition of factor D, the rate limiting step of the alternative pathway, is an attractive approach in inhibiting alternative pathway activation. It should be noted that alternative pathway activation may either directly be triggered or induced by classical or lectin pathway activation for amplification. Whereas inhibition of alternative pathway could block the amplification of complement activation, certain important classical and lectin pathway functions, e.g. C1q or MBL mediated opsonisation and receptor binding, remains intact. In certain conditions of systemic complement activation, such as in ischemia reperfusion injury, trauma or sepsis, the alternative pathway amplification loop may be responsible for an uncontrolled and detrimental activation, irrespective of the initial trigger.

Another strategy relies on targeting specific activation products or their respective receptors, particularly the anaphylotoxins C3a or C5a. In airway hyper-responsiveness, there may be an indication for selective blocking of C3a function, which can be achieved by anti-C3a antibodies or C3aR antagonists, thereby preserving other important C3 functions. Similarly, as C5a is considered the main contributor to the pathophysiology of inflammations associated with systemic complement activation, its function should be selectively blocked, leaving the C5b-9 pathway open for killing of bacteria such as Neisseria.

The main challenge for developing effective and safe anti-complement therapeutics is to balance the beneficial effects obtained by the inhibition with the preservation of sufficient functional activity for anti-microbial protection and for tissue homeostasis.

2.4. Possible adverse effects of complement inhibition

Adverse effects resulting from complement inhibition may be directly related to the function of complement, i.e. increased susceptibility to infection and autoimmune- and immune-complex diseases, arising from impaired opsonisation, adaptive immune response, tolerance or elimination of immune-complexes. To date, such complications have not been observed in animal models. This is best explained by either incomplete inhibition and/or restriction to short-term interventions. In fact, 60% inhibition of complement activity appears to bed sufficient for treatment of collagen-induced arthritis (Wang et al., 1995). This is most important when considering long-term treatment in chronic diseases. A certain degree of inhibition may be sufficient to reduce detrimental effects of complement activation, though defense mechanisms may still be preserved. The risk of infectious complications is most probably highest when blocking C3. The redundancy of the three activation pathways may reduce the risk of infection if only one pathway is selectively blocked. Blocking of C5b-9 formation, however, could lead to increased susceptibility to certain pathogens, such as Neisseriae. Based on experimental studies, Gram-negative septic shock is one of the conditions that may benefit from com-

Samsung Bioepis Exhibit No. 1002
Page 946

plement inhibition. In these cases, treatment with antibiotics would compensate for short-term complement inhibition.

A novel role for complement in tissue regeneration has recently been demonstrated (Mastellos and Lambris, 2002). It is unknown whether impairment of this function will be a consequence of long-term complement inhibition.

Inhibitors which are immunogenic may rapidly loose their efficacy. The risk of immune response is significantly reduced by the use of recombinant human proteins, small molecular inhibitors and humanized antibodies, as discussed below.

2.5. Mode of administration, clearance and long-time treatment

The administration route is critical for patients suffering from chronic diseases requiring long-time therapy. Here, oral, nasal or rectal application is definitely superior to intravenous injection, whereas acute, severe disorders are preferentially treated through the intravenous route. Furthermore, although systemic diseases like sepsis and SIRS may require systemic treatment, diseases with organ specific damage, such as glomerulonephritis, central nervous system diseases and rheumatoid arthritis may benefit from local application. Recombinant proteins and small molecular inhibitors, in contrast to antibodies, generally have a short half-life and are best suited for treatment of acute conditions. Their modifications, e.g. by conjugation to human immunoglobulin Fc fragments, have successfully increased half-life (Harris et al., 2002).

2.6. Targeted application

Targeted application aims at to limit potential systemic side effects. This approach is indicated if the inflammatory process is clearly organ specific and systemic fluid-phase activation can be excluded.

Targeting may be nonspecific, directed to any membrane, or specific, directed to certain cells or organs. One approach for nonspecific targeting is the membrane "tagging", obtained by coupling the inhibitor to a lipid tail. This tail will bind to membranes undergoing internal–external changes, as illustrated by the myristoyl-electrostatic switch paradigm where a truncated form of sCR1 (APT070) was used (Smith and Smith, 2001; Smith, 2002). This agent proved to be 100-fold more potent than the parent molecule and has been used for treatment of experimental rheumatoid arthritis (Linton et al., 2000). Based on the promising experimental results, the agent has been tested in volunteers. Clinical studies in patients with rheumatoid arthritis are underway (Smith, 2002). sCD59 has also been "tagged" in a similar manner, being 100-fold more potent than sCD59 (Smith and Smith, 2001). The rodent complement C3-inhibitor Crry (complement receptor 1 related gene/protein) has been "tagged" using the same approach in experimental animal studies (Fraser et al., 2002).

Another approach for targeted application relies on the antigen-specificity of antibody-conjugates. DAF was successfully targeted to the surface of Chinese hamster ovary cells by a conjugate of DAF and IgG anti-danasyl antibody (Zhang et al., 2001). A similar antigen-targeted form of sCD59 has been produced (Zhang et al., 1999). Recently, conjugates were made between a CR2 fragment, recognizing sites of C3 activation, and DAF and CD59 (Song et al., 2003). These conjugates bound to C3 opsonised cells and were 20-fold more efficient than the untargeted molecules in inhibiting complement activation. In a mouse model of lupus nephritis, the authors showed that CR2-DAF but not soluble DAF bound to the kidney. Direct proximal tubule targeted complement inhibition using single chain antibodies conjugated to complement-inhibitors efficiently attenuated experimental nephrotic syndrome and established a key role for C5b-9 in the pathogenesis (He et al., 2005).

A third approach for targeted inhibition is conjugating the complement-inhibitor to another inflammation-modulating molecule, exemplified by the sCR1-sLe(x) (TP20) molecule (Picard et al., 2000). sLe(x) is a ligand for E- and P-selectin and thus TP20 combines inhibition of complement and leukocyte adhesion (Rittershaus et al., 1999). TP20 was found to be superior to unconjugated sCR1 (TP-10) in reducing tissue damage in an experimental model of cerebral stroke (Huang et al., 1999), in immune-complex mediated lung injury (Mulligan et al., 1999), and in I/R injury in experimental allogenic lung transplantation (Stammberger et al., 2000). Furthermore, TP20 reduced myocardial (Zacharowski et al., 1999) and skeletal muscle I/R injury (Kyriakides et al., 2001a) and moderated acid aspiration injury in mice (Kyriakides et al., 2001b).

2.7. Prodrugs

A prodrug is inactive or has low activity until it reaches a site where it is activated. Although fusion of DAF or CD59 with human immunoglobulin Fc domains markedly extended the half-life (Quigg et al., 1998b; Rehrig et al., 2001; Harris et al., 2002) of the construct, it was found that the biological activity of both regulators was reduced. Full activity was retained upon enzymatic release of the Ig moiety. This principle was utilized to develop a novel DAF fusion protein with virtually no biologic activity. The construct contains a site which is sensible for enzymatic cleavage by a metalloproteinase. After enzymatic cleavage, restricted to sites of inflammation, the biologic activity of DAF was restored (Harris et al., 2003).

2.8. Gene therapy

A transgene strategy for delivery of rat complement regulators using adenovirus vectors has been developed (McGrath et al., 1999). Local production of the complement-inhibitor Crry by astrocytes was found to attenuate experimental allergic encephaloymyelitis (Davoust et al., 1999). sCR1 was delivered by retrovirally transfected cells or by naked DNA directly to the joint and prevented progression of collagen-induced arthritis (Dreja et al., 2000). A similar protective

Samsung Bioepis Exhibit No. 1002
Page 947

effect on antibody-mediated glomerular injury was achieved upon the systemic release of Crry in transgenic mice (Quigg et al., 1998a).

Hyperacute xenotransplant rejection is caused by binding of naturally occurring antibodies and subsequent complement activation. In order to overcome this complement-mediated rejection various transgenic animals expressing human membrane complement regulators have been developed for use in xenotransplantation (Mccurry et al., 1995; Cozzi and White, 1995).

This issue has been extensively described elsewhere.

3. Specific aspects: inhibitors and their application

The complement system is normally kept under strict control by fluid-phase and membrane-bound regulatory proteins. The need for keeping this system under control is illustrated by the fact that there are as many regulators as there are ordinary components, and that deficiency of a regulatory protein is associated with substantially disturbed homeostasis. All complement regulators are inhibitors of activation, except for properdin, which stabilises the alternative C3 convertase (C3bBbP) and thus enhance activation. Many of the candidate drugs for therapeutic complement inhibition are either naturally occurring complement-inhibitory proteins or derivatives thereof.

3.1. Naturally occurring regulators

3.1.1. C1-inhibitor

C1-inhibitor is a naturally occurring serine protease inhibitor and the only known inhibitor of C1r and C1s, and controls MASP-1 and MASP-2 of the lectin pathway as well (Matsushita et al., 2000). Recently, a novel inhibitory function on the alternative pathway was documented (Jiang et al., 2001). Thus, an effect of C1-inhibitor on complement may principally be mediated through either of the initial pathways. Furthermore, the action of C1-inhibitor is not restricted to complement but has a broad spectrum of targets including factor XIIa, kallikrein and factor XIa. C1-inhibitor is available for clinical use as substitution therapy in hereditary angioedema (HAE). Notably, the pathophysiology of HAE is closely related to the release of bradykinin and the main effect of C1-inhibitor is to reduce bradykinin formation through inhibition of the kallikrein/kinin system. From this point of view, HAE is not a complement-mediated disease, and it should be emphasized that the effect of C1-inhibitor, when used for therapeutic treatment, is not necessarily complement-dependent, but may well be explained by inhibition of other proteins. C1-inhibitor has been widely used in a number of clinical conditions. The principle of supra-physiological doses to obtain more efficient regulation has been applied, although in some cases the treatment may be regarded as substitution for acquired low concentrations. Clinical conditions for C1-inhibitor treatment include sepsis, burns, capillary leak syndrome associated with bone marrow transplantation and IL-2 therapy, myocardial infarction, trauma and transplantation (for review, see Kirschfink and Mollnes, 2001). Unfortunately, controlled studies are still missing and except for C1-inhibitor deficiency there is no accepted indication for this treatment.

3.2. Recombinant complement regulatory proteins

The family of regulators of complement activation (RCA) comprises CR1 (CD35), CR2 (CD21), MCP (CD46), DAF (CD55), factor H and C4BP. They are all powerful inhibitors of C3 and C5 convertases, except for CR2, which may play a minor role in regulation of complement. If an extensive inhibition of complement is required, the RCA proteins are candidates since they all interfere with activity of the C3 and C5 convertases.

3.2.1. C4BP and factor H

C4BP and factor H are soluble regulators of the classical and alternative C3/C5 convertases, respectively. Recombinant factor H has been produced (Sharma and Pangburn, 1994). Using a glycosyl-phosphatidyl inositol (GPI) anchor, a membrane form of C4BP was constructed which protected porcine endothelial cells against attack by human complement (Mikata et al., 1998a). A similar approach was used to bind factors H and I to a xenosurface (Yoshitatsu et al., 1999). Binding of factor H to an artificial surface abrogated the surface-induced complement activation and thereby improved biocompatibility (Andersson et al., 2001). C4BP and factor H have not been used clinically.

3.2.2. Crry (complement receptor 1 related gene/protein)

The rodent C3 convertase inhibitor Crry, which has both decay-accelerating and cofactor activity, resembles CR1 and has been used in two different strategies in mouse models. First, a soluble Crry was constructed and coupled to an immunoglobulin tail for injection (Crry-Ig) (Quigg et al., 1998b). Second, Crry was over-expressed as a non-immunogenic soluble protein in the animal under the control of a widely expressed transgene (Quigg et al., 1998a). Both approaches protected mice against experimentally induce acute glomerulonephritis. Furthermore, transgenic expression of Crry in mice developing SLE (MRL/lpr mice) prolonged survival and attenuated renal damage (Bao et al., 2002), later confirmed by treatment with the soluble Crry-Ig protein (Bao et al., 2003). Soluble Crry was also shown to inhibit intestinal I/R injury in mice even when administered 30 min after start of reperfusion (Rehrig et al., 2001). It has recently become evident that control of complement activation is also of crucial importance for the placenta barrier homeostasis where complement activation may induce fetal loss (see Girardi et al., this issue). Thus, in a mouse model of anti-phospholipid antibody-induced fetal loss, soluble Crry was protective (Holers et al., 2002).

Samsung Bioepis Exhibit No. 1002
Page 948

3.2.3. Soluble CR1

CR1 is the only member of the RCA family with cofactor activity and decay-accelerating activity in both the classical and alternative pathway (C4b and C3b). A soluble form of CR1 (sCR1) was constructed by deleting the transmembrane part of the molecule (Weisman et al., 1990). sCR1 inhibited both classical and alternative pathway activation in concentrations equivalent to 1% of physiological C4BP and factor H concentration. The protein was demonstrated to reduce experimental myocardial I/R injury by approximately 50%, a cardioprotective effect which has later been confirmed in several studies (Shandelya et al., 1993; Smith et al., 1993; Homeister et al., 1993; Lazar et al., 1999).

The half-life of the original sCR1 was only a few hours. A slightly extended half-life was obtained by fusing sCR1 with an albumin-binding receptor (Makrides et al., 1996). An sCR1-F(ab')2 chimeric protein combined an extended half-life with improved targeting (Kalli et al., 1991). Its parenteral administration is required.

A variant of sCR1, named sCR(desLHR-A), which specifically inhibits the alternative pathway has been constructed by deleting the C4b binding domain (Scesney et al., 1996). This protein attenuated tissue damage in experimental myocardial infarction (Murohara et al., 1995; Gralinski et al., 1996) and reduced the endothelium-dependent relaxation in rabbit tissue, shown to be mediated by C5b-9 (Lennon et al., 1996).

sCR1 has been widely used in a number of animal disease models with convincing effects on tissue damage. In addition to the beneficial effect on myocardial I/R injury described above, the following I/R injuries have been attenuated by sCR1: local and remote injury in rat intestine (Hill et al., 1992; Eror et al., 1999), gut ischemia and endothelial cell function after hemorrhage and resuscitation in rats (Fruchterman et al., 1998), liver injury (Lehmann et al., 1998; Chavez Cartaya et al., 1995), and local and remote (lung) injury in skeletal muscle (Pemberton et al., 1993; Lindsay et al., 1992).

The beneficial effect of sCR1 in autoimmune diseases has been demonstrated in the passive reverse Arthus reaction, a dermal immune-complex mediated vasculitis (Yeh et al., 1991), in experimental arthritis in rats (Goodfellow et al., 1997, 2000), in immune-complex and complement-mediated lung injuries and thermal trauma in rats (Mulligan et al., 1992). Furthermore, allergic reactions (Lima et al., 1997) and pseudoallergic reaction to infusion of liposomes (Szebeni et al., 1999) were attenuated by sCR1. Improvement was also obtained in an experimental rodent model of ARDS (Rabinovici et al., 1992) as well as in a guinea-pig model of asthma (Regal et al., 1993). In mice, the inflammatory reaction induced by acid aspiration to the lungs was markedly improved by sCR1 (Weiser et al., 1997).

sCR1 has also proved to be efficient in treatment of experimental glomerulonephritis (Couser et al., 1995), myasthenia gravis (Piddlesden et al., 1996), as well as in experimental allergic encephalomyelitis (a model for multiple sclerosis) (Piddlesden et al., 1994), autoimmune neuritis in rats (Jung et al., 1995) and traumatic brain injury (Kaczorowski et al., 1995).

The role of complement in xenotransplantation has been indisputable, and therefore, the effects of sCR1 in reducing hyperacute rejection was not surprising (Pruitt et al., 1991, 1994; Xia et al., 1992; Homeister et al., 1993). Incorporating a mini-CR1 coupled to a GPI anchor into porcine cells was protective against human complement (Mikata et al., 1998b). The role of complement in allotransplant rejection is less clear, but the beneficial effects observed upon sCR1 treatment in various allotransplant models has highlighted complement as an important mechanism in allotransplant rejection as well (Pruitt and Bollinger, 1991; Pratt et al., 1996; Lehmann et al., 1998; Pierre et al., 1998).

Extracorporeal circulation like haemodialysis and cardiopulmonary bypass (CPB) is associated with a complement-dependent systemic inflammatory response. sCR1 reduced lung injury and pulmonary hypertension in pigs undergoing CPB (Gillinov et al., 1993) and inhibited complement and leukocyte activation in a simulated extracorporeal circulation setup (Larsson et al., 1997).

sCR1 has been developed into a pharmaceutical preparation (TP-10; Avant Immunotherapeutics Inc., Needham, MA). It has been tested in patients with ARDS, acute myocardial infarction, lung transplantation and post-cardipulmonary bypass syndrome (Rioux, 2001). The results of the ARDS study showed an efficient inhibition of complement activation, but there was no significant difference between the clinical outcome in the groups (Zimmerman et al., 2000). A pharmacokinetic study of TP-10 in infants undergoing cardiopulmonary bypass showed that administration of the drug was safe and indicated a protective effect on vascular function (Li et al., 2004). In a multi-centre study of patients undergoing cardiopulmonary bypass there was no difference between the test and the placebo group with respect to primary end-point, but a significant reduction in mortality and myocardial infarction was observed in males (Lazar et al., 2004). Interestingly, treatment of lung transplant patients with TP-10 significantly reduced the time on mechanical ventilation compared with placebo (Keshavjee et al., 2005).

3.2.4. Soluble DAF and MCP

A recombinant soluble form of DAF (sDAF) was constructed and found to inhibit complement both in vitro and in vivo. It attenuated the reverse passive Arthus reaction (Moran et al., 1992), but the lack of factor I cofactor activity may limit its potential for clinical application. Similarly, a recombinant soluble form of MCP (sMCP) has been made (Christiansen et al., 1996b). sMCP attenuated the reverse passive Arthus reaction (Christiansen et al., 1996a) and prolonged hyperacute xenograft rejection (Christiansen et al., 1996b). A fusion protein of sMCP and a soluble form of the low affinity human IgG receptor Fc gamma RII (CD32) was more effective in protection against xenotransplant rejection than the sMCP alone (Lanteri et al., 2000). Comparison between sDAF, sMCP and sCR1 showed that sCR1 was more effective than each of the

Samsung Bioepis Exhibit No. 1002
Page 949

other two in the classical pathway. In the alternative pathway, sCR1 and sMCP had almost the same effect (Christiansen et al., 1996a). The combination of sDAF and sMCP was more effective than each protein alone. A fusion protein of sDAF and sMCP was constructed (CAB-2) which retained both decay acceleration (DAF) and co-factor activity (MCP). CAB-2 was protective in the passive reverse Arthus reaction and in Forssman shock (Higgins et al., 1997), and prolonged pig-to-primate heart xenotransplant survival (Salerno et al., 2002) as well as ex vivo porcine-to-human heart transplant graft survival (Kroshus et al., 2000).

3.2.5. Soluble CD59

A recombinant soluble form of CD59, sCD59, has been constructed, but is rather inefficient in the fluid-phase (Sugita et al., 1994). A fusion protein of sCD59 and sDAF has been constructed (Fodor et al., 1995), with the aim of combining a C3/C5 convertase inhibitor activity with a C5b-9 inhibitor. Its possible therapeutic effect remains to be shown. A soluble form of CD59 has been fused with a soluble form of the rodent C3 convertase inhibitor, Crry, enabling inhibition of rodent complement activation both at the C3 and C9 level (Quigg et al., 2000). A membrane-targeted form of sCD59 with biological activity in vitro and in vivo has also been constructed (Fraser et al., 2003). Selective inhibition of C5b-9 may be indicated in certain forms of glomerulonephritides, arthritides and encephalomyelitides which appears depend mainly on the formation of the membrane attack complex.

3.2.6. Microbial proteins

Microbes frequently express proteins with high homology to human complement regulatory proteins and thus protect themselves against complement attack. One such protein, vaccinia virus complement control protein, has CR1-like activity and has been studied in detail (Jha and Kotwal, 2003). It has been suggested as a candidate molecule for complement therapy. In addition to its complement-inhibitory function, this protein also directly interferes with binding of xenoantibodies to their targets.

3.3. Antibodies

Monoclonal antibodies combine the advantage of being highly specific with a relatively long half-life and allow large-scale production. A main limitation is the need for parenteral administration and the risk of immunization. The latter can be reduced by humanizing the antibody, or by producing human monoclonal antibodies, as recently described for an anti-C5 antibody isolated from a human phage display library (Marzari et al., 2002).

3.3.1. Anti-MBL, -factor D and -factor B

Several recent data indicate that the lectin pathway is of importance in the pathogenesis of I/R injury (Jordan et al., 2001; Fiane et al., 2003; Hart et al., 2005). In experimental septicaemia, MBL-A deficiency improved survival (Takahashi et al., 2002). Anti-MBL antibodies blocking the lectin pathway have been developed (Zhao et al., 2002; Collard et al., 2000). As already outlined above, factor D is the rate limiting step in the alternative pathway which is of pivotal importance for the I/R injury in mice (Stahl et al., 2003), either by direct activation or through the amplification loop. An anti-human factor D monoclonal antibody (mAb 166-32) has been developed (Tanhehco et al., 1999; Fung et al., 2001) and found to inhibit complement and leukocyte activation in baboons undergoing CPB (Undar et al., 2002). Blocking of factor B prevented phospholipid-induced pregnancy loss in mice (Thurman et al., 2005).

3.3.2. Anti-C5

Since there are no natural inhibitors for activated C5, there is a particular need to develop specific C5-inhibitors. Furthermore, since C5 may be activated directly, independent of C3, specific inhibitors are required. Monoclonal antibodies to mouse C5, like the mAb BB5.1 (Frei et al., 1987) were important tools to demonstrate the role of the terminal complement pathway in experimental diseases like collagen-induced arthritis (Wang et al., 1995) and lupus-like nephritis (Wang et al., 1996). Notably, such treatment of collagen-induced arthritis with anti-C5 antibody not only prevented disease onset, but also ameliorated established disease. An anti-rat C5 mAb was found to be protective in experimental myocardial infarction (Vakeva et al., 1998). The anti-human C5 antibody N19/8 (Wurzner et al., 1991) was a breakthrough in this field. It was used to demonstrate inhibition of terminal pathway activation in an artificial cardiopulmonary bypass model where leukocyte and platelet activation was also attenuated (Rinder et al., 1995). It also prevented hyperacute graft rejection in a model of porcine-to-human heart transplantation (Kroshus et al., 1995). Later, several anti-C5 antibodies have been generated. One of these, a humanised single chain fragment formerly designated as h5G1.1-scFv (Thomas et al., 1996) has reached clinical trials as pexelizumab (Alexion Pharmaceuticals, Cheshire, CT) with focus on targeting complement in cardiovascular disease. In a study of patients undergoing CPB, complement inhibition was achieved with subsequent reduced leukocyte activation and postoperative complications, including cognitive defects, myocardial damage and blood loss (Fitch et al., 1999). Although no difference in death or myocardial infarction was found between treated and placebo groups, a beneficial effect on their clinical outcomes was observed in a subgroup of patients with isolated coronary artery bypass grafting (Shernan et al., 2004). In a large multi-centre study of patients on cardiopulmonary bypass no difference was found for the same end-points in patients undergoing coronary artery bypass grafting only, but for the whole patient population a significant reduction in death and myocardial infarction 30 days postoperatively was observed for the pexelizumab group (Verrier et al., 2004). A possible beneficial effect of this drug on neurocognitive function after cardiopulmonary bypass was recently reported (Mathew et al., 2004).

Samsung Bioepis Exhibit No. 1002
Page 950

When this compound was used in patients with myocardial infarction, no reduction in infarct size was observed despite an efficient complement-inhibitory effect (Mahaffey et al., 2003). However, a subgroup of myocardial infarction patients with ST-elevation undergoing percutaneous coronary intervention showed a significantly reduced mortality (Granger et al., 2003). Interestingly, CRP and IL-6, both mediators correlating to mortality, were significantly reduced in this patient group upon treatment with pexelizumab (Theroux et al., 2005).

Eculizumab (Alexion Pharmaceuticals, Cheshire, CT), a fully humanized moab C5-inhibitor has been developed for chronic inflammatory (autoimmune) indications, such as rheumatoid arthritis, membraneous glomerulonephritis, dermatomyositis and lupus. Results from ongoing clinical studies are awaited.

This agent has also been tested in patients with paroxysmal nocturnal hemoglubinuria (PNH), a condition with increased complement sensitivity of red cells due to low expression of DAF and CD59. Less haemolysis and requirements for transfusions were observed (Hillmen et al., 2004).

3.3.3. Anti-C5a

C5a is considered the biologically most potent fragment formed during complement activation, and is therefore, regarded as an important target for inhibition. In animal studies, anti-C5a antibodies reduced myocardial I/R injury (Amsterdam et al., 1995), reduced neutrophil-mediated impairment of endothelium-dependent relaxation after CPB (Tofukuji et al., 1998), reduced local and remote injury in intestinal I/R injury (Wada et al., 2001), improved survival (Czermak et al., 1999; Huber-Lang et al., 2001) and reduced IL-6 production (Hopken et al., 1996) and oxygen demand (Mohr et al., 1998) in sepsis. Blocking C5a also inhibited sepsis-related thymic cell apoptosis (Guo et al., 2000; Riedemann et al., 2002), alleviated symptoms of ARDS in septic primates (Stevens et al., 1986) and reduced lung vascular injury following thermal trauma (Schmid et al., 1997). Recently, anti-C5a antibodies were shown to ameliorate impairment of the coagulation and fibrinolytic system in a rat model of sepsis, emphasizing a possible role of C5a in activation of other plasma cascade systems (Laudes et al., 2002).

A novel approach for C5a inhibition was recently demonstrated using a mAb reacting with the C5a moiety of the C5 molecule without inhibiting C5 cleavage (Fung et al., 2003). Thus, C5a is "pre-neutralised" before it is formed and thereby increases efficacy of C5a neutralization. This antibody has been investigated in a human whole blood model of Neisseria infection, with virtually complete inhibition of the inflammatory reaction while bacterial killing was not affected (Sprong et al., 2003).

3.3.4. Anti-C5-9

Anti-C8, which blocks C5b-9 (TCC) formation without interfering with C5 cleavage, was found to reduce tissue damage in rat hearts perfused with human serum (Rollins et al., 1995). Furthermore, anti-C8 inhibited platelet activation during simulated CPB (Rinder et al., 1999).

3.4. Small molecule inhibitors

3.4.1. C1 binding peptides

Peptides interacting with the function of C1q have been produced from phage display libraries (Lauvrak et al., 1997; Roos et al., 2001). Synthetic peptides interfering with IgG binding to C1 prolonged xenograft survival (Fryer et al., 2000). It should be noted that C1q is not only activated by antibodies, but by several other substances which may be of pathogenic importance in human diseases, like beta-amyloid in Alzheimers disease, and C-reactive protein with possible implications for the pathogensis of atherosclerosis. Inhibition of C1q has recently been extensively reviewed (Roos et al., 2002). A novel highly selective small molecule C1s-inhibitor (C1s-INH-248, Knoll, BASF, Germany) was found to protect against experimental myocardial I/R injury (Buerke et al., 2001).

3.4.2. Compstatin

A 13-residue cyclic peptide was isolated using combinatorial peptide libraries to identify C3 binding peptides (Sahu et al., 1996), later characterised in detail and named compstatin (Morikis et al., 1998). This peptide blocks cleavage of C3 and is highly specific for binding to C3. No interaction with other cascade proteins has been demonstrated. It was found to reduce complement and granulocyte activation in an in vitro model of extracorporeal circulation (Nilsson et al., 1998) and to protect against hyperacute xenograft rejection ex vivo (Fiane et al., 1999). Compstatin works only in primates, and therefore, animal studies are limited. One study showed inhibition of heparin–protamine-induced complement activation in a primate in vivo model (Soulika et al., 2000).

3.4.3. C3aR antagonists

A non-peptide C3aR antagonist (SB 290157) blocking human C3aR also antagonises rodent C3aR and was found to reduce neutrophil recruitment in LPS-induced airway neutrophilia (Ames et al., 2001). The C3a/C3aR interaction may be a candidate for therapy in asthma (Bautsch et al., 2000; Drouin et al., 2002). Antibodies neutralizing C3a have also been shown to abolish C3aR mediated function (Elsner et al., 1994). Disrupting the C3aR led to protective anti-inflammatory effects in endotoxin shock (Kildsgaard et al., 2000).

3.4.4. C5aR antagonists

The cyclic peptide AcF[OPdChaWR] (Finch et al., 1999) has been extensively studied in various animal models. It is active in both primates and rodents. Beneficial effects of this antagonist have been observed in I/R injury in small intestine and kidney (Arumugam et al., 2002, 2003) and in inflammatory bowel disease (Woodruff et al., 2003). It attenuated

Samsung Bioepis Exhibit No. 1002
Page 951

chemotaxis and cytokine production (Haynes et al., 2000), endotoxin-induced neutropenia (Short et al., 1999) and the reverse passive Arthus reaction (Strachan et al., 2000). It was effective when given orally to animals with immune-complex mediated dermal inflammation (Strachan et al., 2001) and experimental arthritis (Woodruff et al., 2002). This antagonist also exerted a protective role in anti-phospholipid syndrome with fetal loss (Girardi et al., 2003) and in hepatic I/R injury (Arumugam et al., 2004).

C5aR antagonists were also developed by screening of phage-display libraries and by site directed mutagenesis of C5a, both shown to be effective in reducing complement-mediated inflammation in vivo (Pellas et al., 1998; Heller et al., 1999). The dimeric recombinant human C5a antagonist, CGS 32359 (Pellas et al., 1998), attenuated neutrophil activation and reduced myocardial infarction size in a porcine model of surgical revascularization (Riley et al., 2000). Another C5aR antagonist significantly reduced murine renal I/R injury (deVries et al., 2003). A non-peptide C5aR antagonist active in cynomolgus monkeys and gerbils was shown to inhibit C5a mediated neutropenia after oral administration (Sumichika et al., 2002).

3.5. Other inhibitors

Numerous natural and synthetic substances have been documented to inhibit complement, but these are in general not complement specific. Examples are nafamostat mesilate (FUTHAN; FUT-175), aprotinin, K-76 monocarboxylic acid (MX-1) and heparin. The latter has a number of effects on complement and coagulation and modifications to obtain complement-inhibitory heparin without effect on coagulation have been made (Weiler et al., 1992). Dextran potentiates the effects several complement-inhibitory proteins including C1-inhibitor and was found to delay ex vivo hyperacute xenograft rejection (Fiorante et al., 2001). Coating of artificial devises with heparin improves biocompatibility by reducing complement activation and subsequent inflammatory reaction (Olsson et al., 2000).

High-dose intravenous immunoglobulins (IVIG) are currently used to treat various inflammatory diseases. The list of biological effects of IVIG is long, including several complement modulating activities. In fact, the effect of IVIG on complement is not by mediated by inhibition of complement activation. IVIG act as scavenger for C1q, C4b and C3b binding, thus diverting the activation products from the target to the immunoglobulin molecules (Frank et al., 2000). Thus, IVIG should not be used as a primary complement-inhibitor, but its effect on complement could be an additional benefit to certain inflammatory diseases.

Cobra venom factor (CVF) traditionally used for experimental complement depletion is frequently on the list of potential therapeutic complement-inhibitors. It should be noted, however, that CVF is not a complement-inhibitor, but a potent complement activator, which depletes C3 from plasma by activating the alternative pathway amplification loop. The activation per se may influence the results obtained, therefore, specific complement-inhibitors developed to date should replace CVF in animal studies.

4. Concluding remarks

Three decades ago the role of complement in human disease was largely unknown. Two decades ago, it became evident that complement activation was associated with a number of pathophysiologic conditions, although the role of this activation in the pathogenesis of the diseases was largely unknown. During the last decade studies using specific complement-inhibitors or complement knock-out animals have revealed that complement plays a crucial role in the pathogenesis of inflammatory tissue destruction. Based on these encouraging data, a great enthusiasm evolved for specific complement inhibition as a novel treatment of human diseases. Unfortunately, the progress towards a clinical application has been relatively slow. This may be attributed to several reasons: human diseases appear to be more complex in their pathophysiology than reflected by those induced in animal models. Experimental conditions differ significantly from those in the clinic with respect to time for and mode of application. The number of drugs which meanwhile reached the stage of clinical trials is markedly limited compared to those initially and still used in animal models. Finally, the selection of patients and size of test groups for a chosen indication may have been inadequate for a clear demonstration of a clinical benefit. There is still a need for more experimental studies to further elucidate the mechanisms of complement-mediated damage. This will provide a rational approach to design and optimise the treatment, i.e. identifying the component(s) needed to be inhibited in the actual condition and targeting the inhibition to the actual site of inflammation. The fate of this exciting new avenue in antiphlogistic therapy will depend not only on the development of novel complement-inhibitors, but also on the success of those which are already advanced to clinical trials.

References

Ames, R.S., Lee, D., Foley, J.J., Jurewicz, A.J., Tornetta, M.A., Bautsch, W., Settmacher, B., Klos, A., Erhard, K.F., Cousins, R.D., Sulpizio, A.C., Hieble, J.P., McCafferty, G., Ward, K.W., Adams, J.L., Bondinell, W.E., Underwood, D.C., Osborn, R.R., Badger, A.M., Sarau, H.M., 2001. Identification of a selective nonpeptide antagonist of the anaphylatoxin C3a receptor that demonstrates antiinflammatory activity in animal models. J. Immunol. 166, 6341–6348.

Amsterdam, E.A., Stahl, G.L., Pan, H.L., Rendig, S.V., Fletcher, M.P., Longhurst, J.C., 1995. Limitation of reperfusion injury by a monoclonal antibody to C5a during myocardial infarction in pigs. Am. J. Physiol. Heart Circ. Physiol. 37, H448–H457.

Andersson, J., Larsson, R., Richter, R., Ekdahl, K.N., Nilsson, B., 2001. Binding of a model regulator of complement activation (RCA) to a biomaterial surface: surface-bound factor H inhibits complement activation. Biomaterials 22, 2435–2443.

Samsung Bioepis Exhibit No. 1002
Page 952

Arumugam, T.V., Shiels, I.A., Strachan, A.J., Abbenante, G., Fairlie, D.P., Taylor, S.M., 2003. A small molecule C5a receptor antagonist protects kidneys from ischemia/reperfusion injury in rats. Kidney Int. 63, 134–142.

Arumugam, T.V., Shiels, I.A., Woodruff, T.M., Reid, R.C., Fairlie, D.P., Taylor, S.M., 2002. Protective effect of a new C5a receptor antagonist against ischemia–reperfusion injury in the rat small intestine. J. Surg. Res. 103, 260–267.

Arumugam, T.V., Woodruff, T.M., Stocks, S.Z., Proctor, L.M., Pollitt, S., Shiels, I.A., Reid, R.C., Fairlie, D.P., Taylor, S.M., 2004. Protective effect of a human C5a receptor antagonist against hepatic ischaemia–reperfusion injury in rats. J. Hepatol. 40, 934–941.

Asghar, S.S., Pasch, M.C., 2000. Therapeutic inhibition of the complement system. Y2K update. Front. Biosci. 5, E63–E82.

Bao, L.H., Haas, M., Boackle, S.A., Kraus, D.M., Cunningham, P.N., Park, P., Alexander, J.J., Anderson, R.K., Culhane, K., Holers, V.M., Quigg, R.J., 2002. Transgenic expression of a soluble complement inhibitor protects against renal disease and promotes survival in MRL/lpr mice. J. Immunol. 168, 3601–3607.

Bao, L.H., Haas, M., Kraus, D.M., Hack, B.K., Rakstang, J.K., Holers, V.M., Quigg, R.J., 2003. Administration of a soluble recombinant complement C3 inhibitor protects against renal disease in MRL/lpr mice. J. Am. Soc. Nephrol. 14, 670–679.

Bautsch, W., Hoymann, H.G., Zhang, Q.W., MeierWiedenbach, I., Raschke, U., Ames, R.S., Sohns, B., Flemme, N., zuVilsendorf, A.M., Grove, M., Klos, A., Kohl, J., 2000. Cutting edge: guinea pigs with a natural C3a-receptor defect exhibit decreased bronchoconstriction in allergic airway disease: evidence for an involvement of the C3a anaphylatoxin in the pathogenesis of asthma. J. Immunol. 165, 5401–5405.

Buerke, M., Schwertz, H., Seitz, W., Meyer, J., Darius, H., 2001. Novel small molecule inhibitor of C1s exerts cardioprotective effects in ischemia–reperfusion injury in rabbits. J. Immunol. 167, 5375–5380.

Chavez Cartaya, R.E., Desola, G.P., Wright, L., Jamieson, N.V., White, D.J.G., 1995. Regulation of the complement cascade by soluble complement receptor type 1. Protective effect in experimental liver ischemia and reperfusion. Transplantation 59, 1047–1052.

Christiansen, D., Milland, J., Thorley, B.R., Mckenzie, I.F.C., Loveland, B.E., 1996a. A functional analysis of recombinant soluble CD46 in vivo and a comparison with recombinant soluble forms of CD55 and CD35 in vitro. Eur. J. Immunol. 26, 578–585.

Christiansen, D., Milland, J., Thorley, B.R., Mckenzie, I.F.C., Mottram, P.L., Purcell, L.J., Loveland, B.E., 1996b. Engineering of recombinant soluble CD46: an inhibitor of complement activation. Immunology 87, 348–354.

Collard, C.D., Vakeva, A., Morrissey, M.A., Agah, A., Rollins, S.A., Reenstra, W.R., Buras, J.A., Meri, S., Stahl, G.L., 2000. Complement activation after oxidative stress—role of the lectin complement pathway. Am. J. Pathol. 156, 1549–1556.

Couser, W.G., Johnson, R.J., Young, B.A., Yeh, C.G., Toth, C.A., Rudolph, A.R., 1995. The effects of soluble recombinant complement receptor 1 on complement-mediated experimental glomerulonephritis. J. Am. Soc. Nephrol. 5, 1888–1894.

Cozzi, E., White, D.J.G., 1995. The generation of transgenic pigs as potential organ donors for humans. Nat. Med. 1, 964–966.

Czermak, B.J., Sarma, V., Pierson, C.L., Warner, R.L., HuberLang, M., Bless, N.M., Schmal, H., Friedl, H.P., Ward, P.A., 1999. Protective effects of C5a blockade in sepsis. Nat. Med. 5, 788–792.

Davoust, N., Nataf, S., Reiman, R., Holers, M.V., Campbell, I.L., Barnum, S.R., 1999. Central nervous system-targeted expression of the complement inhibitor sCrry prevents experimental allergic encephalomyelitis. J. Immunol. 163, 6551–6556.

deVries, B., Kohl, J., Leclercq, W.K.G., Wolfs, T.G.A.M., VanBijnen, A.A.J.H., Heeringa, P., Buurman, W.A., 2003. Complement factor C5a mediates renal ischemia–reperfusion injury independent from neutrophils. J. Immunol. 170, 3883–3889.

Dreja, H., Annenkov, A., Chernajovsky, Y., 2000. Soluble complement receptor 1 (CD35) delivered by retrovirally infected syngeneic cells or by naked DNA injection prevents the progression of collagen-induced arthritis. Arthritis Rheum. 43, 1698–1709.

Drouin, S.M., Corry, D.B., Hollman, T.J., Kildsgaard, J., Wetsel, R.A., 2002. Absence of the complement anaphylatoxin C3a receptor suppresses Th2 effector functions in a murine model of pulmonary allergy. J. Immunol. 169, 5926–5933.

Elsner, J., Oppermann, M., Czech, W., Kapp, A., 1994. C3a activates the respiratory burst in human polymorphonuclear neutrophilic leukocytes via pertussis toxin-sensitive G-proteins. Blood 83, 3324–3331.

Eror, A.T., Stojadinovic, A., Starnes, B.W., Makrides, S.C., Tsokos, G.C., SheaDonohue, T., 1999. Antiinflammatory effects of soluble complement receptor type 1 promote rapid recovery of ischemia/reperfusion injury in rat small intestine. Clin. Immunol. 90, 266–275.

Fiane, A.E., Mollnes, T.E., Videm, V., Hovig, T., Hogasen, K., Mellbye, O.J., Spruce, L., Moore, W.T., Sahu, A., Lambris, J.D., 1999. Compstatin, a peptide inhibitor of C3, prolongs survival of ex vivo perfused pig xenografts. Xenotransplantation 6, 52–65.

Fiane, A.E., Videm, V., Lingaas, P.S., Heggelungd, L., Nielsen, E.W., Geiran, O.R., Fung, M., Mollnes, T.E., 2003. Mechanism of complement activation and its role in the inflammatory response following thoraco-abdominal aortic aneurysm repair. Circulation 108, 849–856.

Finch, A.M., Wong, A.K., Paczkowski, N.J., Wadi, S.K., Craik, D.J., Fairlie, D.P., Taylor, S.M., 1999. Low-molecular-weight peptidic and cyclic antagonists of the receptor for the complement factor C5a. J. Med. Chem. 42, 1965–1974.

Fiorante, P., Banz, Y., Mohacsi, P.J., Kappeler, A., Wuillemin, W.A., Macchiarini, P., Roos, A., Daha, M.R., Schaffner, T., Haeberli, A., Mazmanian, G.M., Rieben, R., 2001. Low molecular weight dextran sulfate prevents complement activation and delays hyperacute rejection in pig-to-human xenotransplantation models. Xenotransplantation 8, 24–35.

Fitch, J.C.K., Rollins, S., Matis, L., Alford, B., Aranki, S., Collard, C.D., Dewar, M., Elefteriades, J., Hines, R., Kopf, G., Kraker, P., Li, L., OHara, R., Rinder, C., Rinder, H., Shaw, R., Smith, B., Stahl, G., Shernan, S.K., 1999. Pharmacology and biological efficacy of a recombinant, humanized, single-chain antibody C5 complement inhibitor in patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass. Circulation 100, 2499–2506.

Fodor, W.L., Rollins, S.A., Guilmette, E.R., Setter, E., Squinto, S.P., 1995. A novel bifunctional chimeric complement inhibitor that regulates C3 convertase and formation of the membrane attack complex. J. Immunol. 155, 4135–4138.

Frank, M.M., Miletic, V.D., Jiang, H., 2000. Immunoglobulin in the control of complement action. Immunol. Res. 22, 137–146.

Fraser, D.A., Harris, C.L., Smith, R.A.G., Morgan, B.P., 2002. Bacterial expression and membrane targeting of the rat complement regulator Crry: a new model anticomplement therapeutic. Protein Sci. 11, 2512–2521.

Fraser, D.A., Harris, C.L., Williams, A.S., Mizuno, M., Gallagher, S., Smith, R.A.G., Morgan, B.P., 2003. Generation of a recombinant, membrane-targeted form of the complement regulator CD59—activity in vitro and in vivo. J. Biol. Chem. 278, 48921–48927.

Frei, Y., Lambris, J.D., Stockinger, B., 1987. Generation of a monoclonal antibody to mouse C5 application in an ELISA assay for detection of anti-C5 antibodies. Mol. Cell. Probes 1, 141–149.

Fruchterman, T.M., Spain, D.A., Wilson, M.A., Harris, P.D., Garrison, R.N., 1998. Complement inhibition prevents gut ischemia and endothelial cell dysfunction after hemorrhage/resuscitation. Surgery 124, 782–792.

Fryer, J.P., Leventhal, J.R., Pao, W., Stadler, C., Jones, M., Walsh, T., Zhong, R., Zhang, Z., Wang, H., Goodman, D.J., Kurek, M., Dapice, A.J.F., Blondin, B., Ivancic, D., Buckingham, F., Kaufman, D., Abecassis, M., Stuart, F., Anderson, B.E., 2000. Synthetic peptides which inhibit the interaction between C1q and immunoglobulin and prolong xenograft survival. Transplantation 70, 828–836.

Samsung Bioepis Exhibit No. 1002
Page 953

Fung, M., Loubser, P.G., Undar, A., Mueller, M., Sun, C., Sun, W.N., Vaughn, W.K., Fraser Jr., C.D., 2001. Inhibition of complement, neutrophil, and platelet activation by an anti-factor D monoclonal antibody in simulated cardiopulmonary bypass circuits. J. Thorac. Cardiovasc. Surg. 122, 113–122.

Fung, M., Lu, M., Fure, H., Sun, W., Sun, C., Shi, N.Y., Du, Y., Su, J., Swanson, X., Mollnes, T.E., 2003. Pre-neutralization of C5a-mediated effects by the monoclonal antibody 137-26 reacting with the C5a moiety of native C5 without preventing C5 cleavage. Clin. Exp. Immunol. 133, 160–169.

Gillinov, A.M., Devaleria, P.A., Winkelstein, J.A., Wilson, I., Curtis, W.E., Shaw, D., Yeh, C.G., Rudolph, A.R., Baumgartner, W.A., Herskowitz, A., Cameron, D.E., 1993. Complement inhibition with soluble complement receptor type 1 in cardiopulmonary bypass. Ann. Thorac. Surg. 55, 619–624.

Girardi, G., Berman, J., Redecha, P., Spruce, L., Thurman, J.M., Kraus, D., Hollmann, T.J., Casali, P., Caroll, M.C., Wetsel, R.A., Lambris, J.D., Holers, M.V., Salmon, J.E., 2003. Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome. J. Clin. Invest. 112, 1644–1654.

Goodfellow, R.M., Williams, A.S., Levin, J.L., Williams, B.D., Morgan, B.P., 2000. Soluble complement receptor one (SCR1) inhibits the development and progression of rat collagen-induced arthritis. Clin. Exp. Immunol. 119, 210–216.

Goodfellow, R.M., Williams, A.S., Levin, J.L., Williams, B.D., Morgan, B.P., 1997. Local therapy with soluble complement receptor 1 (sCR1) suppresses inflammation in rat mono-articular arthritis. Clin. Exp. Immunol. 110, 45–52.

Gralinski, M.R., Wiater, B.C., Assenmacher, A.N., Lucchesi, B.R., 1996. Selective inhibition of the alternative complement pathway by sCR1[desLHR-A] protects the rabbit isolated heart from human complement-mediated damage. Immunopharmacology 34, 79–88.

Granger, C.B., Mahaffey, K.W., Weaver, W.D., Theroux, P., Hochman, J.S., Filloon, T.G., Rollins, S., Todaro, T.G., Nicolau, J.C., Ruzyllo, W., Armstrong, P.W., 2003. Pexelizumab, an anti-C5 complement antibody, as adjunctive therapy to primary percutaneous coronary intervention in acute myocardial infarction—the complement inhibition in myocardial infarction treated with angioplasty (COMMA) trial. Circulation 108, 1184–1190.

Guo, R.F., HuberLang, M., Wang, X., Sarma, V., Padgaonkar, V.A., Craig, R.A., Riedemann, N.C., McClintock, S.D., Hlaing, T., Shi, M.M., Ward, P.A., 2000. Protective effects of anti-C5a in sepsis-induced thymocyte apoptosis. J. Clin. Invest. 106, 1271–1280.

Harris, C.L., Hughes, C.E., Williams, A.S., Goodfellow, I., Evans, D.J., Caterson, B., Morgan, B.P., 2003. Generation of anti-complement 'prodrugs': cleavable reagents for specific delivery of complement regulators to disease sites. J. Biol. Chem. 278, 36068–36076.

Harris, C.L., Williams, A.S., Linton, S.M., Morgan, B.P., 2002. Coupling complement regulators to immunoglobulin domains generates effective anti-complement reagents with extended half-life in vivo. Clin. Exp. Immunol. 129, 198–207.

Hart, M.L., Ceonzo, K.A., Shaffer, L.A., Takahashi, K., Rother, R.P., Reenstra, W.R., Buras, J.A., Stahl, G.L., 2005. Gastrointestinal ischemia–reperfusion injury is lectin complement pathway dependent without involving C1q. J. Immunol. 174, 6373–6380.

Haynes, D.R., Harkin, D.G., Bignold, L.P., Hutchens, M.J., Taylor, S.M., Fairlie, D.P., 2000. Inhibition of C5a-induced neutrophil chemotaxis and macrophage cytokine production in vitro by a new C5a receptor antagonist. Biochem. Pharmacol. 60, 729–733.

He, C., Imai, M., Song, H., Quigg, R.J., Tomlinson, S., 2005. Complement inhibitors targeted to the proximal tubule prevent injury in experimental nephrotic syndrome and demonstrate a key role for c5b-9. J. Immunol. 174, 5750–5757.

Heller, T., Hennecke, M., Baumann, U., Gessner, J.E., Vilsendorf, A.M.Z., Baensch, M., Boulay, F., Kola, A., Klos, A., Bautsch, W., Kohl, J., 1999. Selection of a C5a receptor antagonist from phage libraries attenuating the inflammatory response in immune complex disease and ischemia reperfusion injury. J. Immunol. 163, 985–994.

Higgins, P.J., Ko, J.L., Lobell, R., Sardonini, C., Alessi, M.K., Yeh, C.G., 1997. A soluble chimeric complement inhibitory protein that possesses both decay-accelerating and factor I cofactor activities. J. Immunol. 158, 2872–2881.

Hill, J., Lindsay, T.F., Ortiz, F., Yeh, C.G., Hechtman, H.B., Moore, F.D., 1992. Soluble complement receptor type-1 ameliorates the local and remote organ injury after intestinal ischemia–reperfusion in the rat. J. Immunol. 149, 1723–1728.

Hillmen, P., Hall, C., Marsh, J.C., Elebute, M., Bombara, M.P., Petro, B.E., Cullen, M.J., Richards, S.J., Rollins, S.A., Mojcik, C.F., Rother, R.P., 2004. Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. N. Engl. J. Med. 350, 552–559.

Holers, V.M., Girardi, G., Mo, L., Guthridge, J.M., Molina, H., Pierangeli, S.S., Espinola, R., Xiaowei, L.E., Mao, D.L., Vialpando, C.G., Salmon, J.E., 2002. Complement C3 activation is required for antiphospholipid antibody-induced fetal loss. J. Exp. Med. 195, 211–220.

Homeister, J.W., Satoh, P.S., Kilgore, K.S., Lucchesi, B.R., 1993. Soluble complement receptor type-1 prevents human complement-mediated damage of the rabbit isolated heart. J. Immunol. 150, 1055–1064.

Hopken, U., Mohr, M., Struber, A., Montz, H., Burchardi, H., Gotze, O., Oppermann, M., 1996. Inhibition of interleukin-6 synthesis in an animal model of septic shock by anti-C5a monoclonal antibodies. Eur. J. Immunol. 26, 1103–1109.

Huang, J., Kim, L.J., Mealey, R., Marsh, H.C., Zhang, Y., Tenner, A.J., Connolly, E.S., Pinsky, D.J., 1999. Neuronal protection in stroke by an sLe(X)-glycosylated complement inhibitory protein. Science 285, 595–599.

Huber-Lang, M.S., Sarma, J.V., McGuire, S.R., Lu, K.T., Guo, R.F., Padgaonkar, V.A., Younkin, E.M., Laudes, I.J., Riedemann, N.C., Younger, J.G., Ward, P.A., 2001. Protective effects of anti-C5a peptide antibodies in experimental sepsis. FASEB J. 15, 568–570.

Jha, P., Kotwal, G.J., 2003. Vaccinia complement control protein: multifunctional protein and a potential wonder drug. J. Biosci. 28, 265–271.

Jiang, H., Wagner, E., Zhang, H.M., Frank, M.M., 2001. Complement 1 inhibitor is a regulator of the alternative complement pathway. J. Exp. Med. 194, 1609–1616.

Jordan, J.E., Montalto, M.C., Stahl, G.L., 2001. Inhibition of mannose-binding lectin reduces postischemic myocardial reperfusion injury. Circulation 104, 1413–1418.

Jung, S., Toyka, K.V., Hartung, H.P., 1995. Soluble complement receptor type 1 inhibits experimental autoimmune neuritis in Lewis rats. Neurosci. Lett. 200, 167–170.

Kaczorowski, S.L., Schiding, J.K., Toth, C.A., Kochanek, P.M., 1995. Effect of soluble complement receptor-1 on neutrophil accumulation after traumatic brain injury in rats. J. Cereb. Blood Flow Metab. 15, 860–864.

Kalli, K.R., Hsu, P.H., Bartow, T.J., Ahearn, J.M., Matsumoto, A.K., Klickstein, L.B., Fearon, D.T., 1991. Mapping of the C3b-binding site of CR1 and construction of a (CR1)2-F(ab')2 chimeric complement inhibitor. J. Exp. Med. 174, 1451–1460.

Keshavjee, S., Davis, R.D., Zamora, M.R., de Perrot, M., Patterson, G.A., 2005. A randomized, placebo-controlled trial of complement inhibition in ischemia–reperfusion injury after lung transplantation in human beings. J. Thorac. Cardiovasc. Surg. 129, 423–428.

Kildsgaard, J., Hollmann, T.J., Matthews, K.W., Bian, K., Murad, F., Wetsel, R.A., 2000. Cutting edge: targeted disruption of the C3a receptor gene demonstrates a novel protective anti-inflammatory role for C3a in endotoxin-shock. J. Immunol. 165, 5406–5409.

Kirschfink, M., 2001. Targeting complement in therapy. Immunol. Rev. 180, 177–189.

Kirschfink, M., Mollnes, T.E., 2001. C1-inhibitor: an anti-inflammatory reagent with therapeutic potential. Expert Opin. Pharmacother. 2, 1073–1084.

Samsung Bioepis Exhibit No. 1002
Page 954

Kroshus, T.J., Rollins, S.A., Dalmasso, A.P., Elliott, E.A., Matis, L.A., Squinto, S.P., Bolman, R.M., 1995. Complement inhibition with an anti-C5 monoclonal antibody prevents acute cardiac tissue injury in an ex vivo model of pig-to-human xenotransplantation. Transplantation 60, 1194–1202.

Kroshus, T.J., Salerno, C.T., Yeh, C.G., Higgins, P.J., Bolman, R.M., Dalmasso, A.P., 2000. A recombinant soluble chimeric complement inhibitor composed of human CD46 and CD55 reduces acute cardiac tissue injury in models of pig-to-human heart transplantation. Transplantation 69, 2282–2289.

Kyriakides, C., Wang, Y., Austen, W.G., Favuzza, J., Kobzik, L., Moore, F.D., Hechtman, H.B., 2001b. Sialyl Lewis(X) hybridized complement receptor type 1 moderates acid aspiration injury. Am. J. Physiol. Lung Cell. Mol. Physiol. 281, L1494–L1499.

Kyriakides, C., Wang, Y., Austen, W.G., Favuzza, J., Kobzik, L., Moore, F.D., Hechtman, H.B., 2001a. Moderation of skeletal muscle reperfusion injury by a sLe(X)-glycosylated complement inhibitory protein. Am. J. Physiol. Cell. Physiol. 281, C224–C230.

Lambris, J.D., Holers, V.M. (Eds.), 2000. Therapeutic Intervention in the Complement System. Humana Press, Totowa, NJ.

Lanteri, M.B., Powell, M.S., Christiansen, D., Li, Y.Q., Hogarth, P.M., Sandrin, M.S., Mckenzie, I.F.C., Loveland, B.E., 2000. Inhibition of hyperacute transplant rejection by soluble proteins with the functional domains of CD46 and Fc gamma RII. Transplantation 69, 1128–1136.

Larsson, R., Elgue, G., Larsson, A., Ekdahl, K.N., Nilsson, U.R., Nilsson, B., 1997. Inhibition of complement activation by soluble recombinant CR1 under conditions resembling those in a cardiopulmonary circuit: reduced up-regulation of CD11b and complete abrogation of binding of PMNs to the biomaterial surface. Immunopharmacology 38, 119–127.

Laudes, I.J., Chu, J.C., Sikranth, S., HuberLang, M., Guo, R.F., Riedemann, N., Sarma, J.V., Schmaier, A.H., Ward, P.A., 2002. Anti-C5a ameliorates coagulation/fibrinolytic protein changes in a rat model of sepsis. Am. J. Pathol. 160, 1867–1875.

Lauvrak, V., Brekke, O.H., Ihle, O., Lindqvist, B.H., 1997. Identification and characterisation of C1q-binding phage displayed peptides. Biol. Chem. 378, 1509–1519.

Lazar, H.L., Bao, Y.S., Gaudiani, J., Rivers, S., Marsh, H., 1999. Total complement inhibition—an effective strategy to limit ischemic injury during coronary revascularization on cardiopulmonary bypass. Circulation 100, 1438–1442.

Lazar, H.L., Bokesch, P.M., Van Lenta, F., Fitzgerald, C., Emmett, C., Marsh Jr., H.C., Ryan, U., 2004. Soluble human complement receptor 1 limits ischemic damage in cardiac surgery patients at high risk requiring cardiopulmonary bypass. Circulation 110, II274–II279.

Lehmann, T.G., Koeppel, T.A., Kirschfink, M., Gebhard, M.M., Herfarth, C., Otto, G., Post, S., 1998. Complement inhibition by soluble complement receptor type 1 improves microcirculation after rat liver transplantation. Transplantation 66, 717–722.

Lennon, P.F., Collard, C.D., Morrissey, M.A., Stahl, G.L., 1996. Complement-induced endothelial dysfunction in rabbits: mechanisms, recovery, and gender differences. Am. J. Physiol. Heart Circ. Physiol. 39, H1924–H1932.

Li, J.S., Sanders, S.P., Perry, A.E., Stinnett, S.S., Jaggers, J., Bokesch, P., Reynolds, L., Nassar, R., Anderson, P.A., 2004. Pharmacokinetics and safety of TP10, soluble complement receptor 1, in infants undergoing cardiopulmonary bypass. Am. Heart J. 147, 173–180.

Lima, M.C., Prouvost-Danon, A., Silva, P.M., Chagas, M.S., Calheiros, A.S., Cordeiro, R.S., Latine, D., Bazin, H., Ryan, U.S., Martins, M.A., 1997. Studies on the mechanisms involved in antigen-evoked pleural inflammation in rats: contribution of IgE and complement. J. Leukoc. Biol. 61, 286–292.

Lindsay, T.F., Hill, J., Ortiz, F., Rudolph, A., Valeri, C.R., Hechtman, H.B., Moore, F.D., 1992. Blockade of complement activation prevents local and pulmonary albumin leak after lower torso ischemia–reperfusion. Ann. Surg. 216, 677–683.

Linton, S.M., Williams, A.S., Dodd, I., Smith, R., Williams, B.D., Morgan, B.P., 2000. Therapeutic efficacy of a novel membrane-targeted complement regulator in antigen-induced arthritis in the rat. Arthritis Rheum. 43, 2590–2597.

Mahaffey, K.W., Granger, C.B., Nicolau, J.C., Ruzyllo, W., Weaver, W.D., Theroux, P., Hochman, J.S., Filloon, T.G., Mojcik, C.F., Todaro, T.G., Armstrong, P.W., 2003. Effect of pexelizumab, an anti-C5 complement antibody, as adjunctive therapy to fibrinolysis in acute myocardial infarction—the complement inhibition in myocardial infarction treated with thrombolytics (COMPLY) trial. Circulation 108, 1176–1183.

Makrides, S.C., Nygren, P.A., Andrews, B., Ford, P.J., Evans, K.S., Hayman, E.G., Adari, H., Levin, J., Uhlen, M., Toth, C.A., 1996. Extended in vivo half-life of human soluble complement receptor type 1 fused to a serum albumin-binding receptor. J. Pharmacol. Exp. Ther. 277, 534–542.

Marzari, R., Sblattero, D., Macor, P., Fischetti, F., Gennaro, R., Marks, J.D., Bradbury, A., Tedesco, F., 2002. The cleavage site of C5 from man and animals as a common target for neutralizing human monoclonal antibodies: in vitro and in vivo studies. Eur. J. Immunol. 32, 2773–2782.

Mastellos, D., Lambris, J.D., 2002. Complement: more than a 'guard' against invading pathogens? Trends Immunol. 23, 485–491.

Mathew, J.P., Shernan, S.K., White, W.D., Fitch, J.C., Chen, J.C., Bell, L., Newman, M.F., 2004. Preliminary report of the effects of complement suppression with pexelizumab on neurocognitive decline after coronary artery bypass graft surgery. Stroke 35, 2335–2339.

Matsushita, M., Thiel, S., Jensenius, J.C., Terai, I., Fujita, T., 2000. Proteolytic activities of two types of mannose-binding lectin-associated serine protease. J. Immunol. 165, 2637–2642.

Mccurry, K.R., Kooyman, D.L., Alvarado, C.G., Cotterell, A.H., Martin, M.J., Logan, J.S., Platt, J.L., 1995. Human complement regulatory proteins protect swine-to-primate cardiac xenografts from humoral injury. Nat. Med. 1, 423–427.

McGrath, Y., Wilkinson, G.W.G., Spiller, O.B., Morgan, B.P., 1999. Development of adenovirus vectors encoding rat complement regulators for use in therapy in rodent models of inflammatory diseases. J. Immunol. 163, 6834–6840.

Mikata, S., Miyagawa, S., Iwata, K., Nagasawa, S., Hatanaka, M., Matsumoto, M., Kamiike, W., Matsuda, H., Shirakura, R., Seya, T., 1998a. Regulation of complement-mediated swine endothelial cell lysis by a surface-bound form of human C4b binding protein. Transplantation 65, 363–368.

Mikata, S., Miyagawa, S., Yoshitatsu, M., Ikawa, M., Okabe, M., Matsuda, H., Shirakura, R., 1998b. Prevention of hyperacute rejection by phosphatidylinositol-anchored mini-complement receptor type 1. Transpl. Immunol. 6, 107–110.

Mohr, M., Hopken, U., Oppermann, M., Mathes, C., Goldmann, K., Siever, S., Gotze, O., Burchardi, H., 1998. Effects of anti-C5a monoclonal antibodies on oxygen use in a porcine model of severe sepsis. Eur. J. Clin. Invest. 28, 227–234.

Mollnes, T.E., 2004. Therapeutic manipulation of the complement system. In: Szebeni, J. (Ed.), The Complement System: Novel Roles in Health and Disease. Kluwer Academic Publishers, Massachusetts, pp. 483–516.

Mollnes, T.E., Song, W.C., Lambris, J.D., 2002. Complement in inflammatory tissue damage and disease. Trends Immunol. 23, 61–64.

Moran, P., Beasley, H., Gorrell, A., Martin, E., Gribling, P., Fuchs, H., Gillett, N., Burton, L.E., Caras, I.W., 1992. Human recombinant soluble decay-accelerating factor inhibits complement activation in vitro and in vivo. J. Immunol. 149, 1736–1743.

Morgan, B.P., Harris, C.L., 2003. Complement therapeutics; history and current progress. Mol. Immunol. 40, 159–170.

Morikis, D., AssaMunt, N., Sahu, A., Lambris, J.D., 1998. Solution structure of compstatin, a potent complement inhibitor. Protein Sci. 7, 619–627.

Mulligan, M.S., Warner, R.L., Rittershaus, C.W., Thomas, L.J., Ryan, U.S., Foreman, K.E., Crouch, L.D., Till, G.O., Ward, P.A., 1999.

Samsung Bioepis Exhibit No. 1002
Page 955

Endothelial targeting and enhanced antiinflammatory effects of complement inhibitors possessing sialyl Lewis(X) moieties. J. Immunol. 162, 4952–4959.

Mulligan, M.S., Yeh, C.G., Rudolph, A.R., Ward, P.A., 1992. Protective effects of soluble CR1 in complement-mediated and neutrophil-mediated tissue injury. J. Immunol. 148, 1479–1485.

Murohara, T., Guo, J.P., Delyani, J.A., Lefer, A.M., 1995. Cardioprotective effects of selective inhibition of the two complement activation pathways in myocardial ischemia and reperfusion injury. Methods Find. Exp. Clin. Pharmacol. 17, 499–507.

Nilsson, B., Larsson, R., Hong, J., Elgue, G., Ekdahl, K.N., Sahu, A., Lambris, J.D., 1998. Compstatin inhibits complement and cellular activation in whole blood in two models of extracorporeal circulation. Blood 92, 1661–1667.

Olsson, P., Sanchez, J., Mollnes, T.E., Riesenfeld, J., 2000. On the blood compatibility of end-point immobilized heparin. J. Biomater. Sci. Polym. Ed. 11, 1261–1273.

Pellas, T.C., Boyar, W., van Oostrum, J., Wasvary, J., Fryer, L.R., Pastor, G., Sills, M., Braunwalder, A., Yarwood, D.R., Kramer, R., Kimble, E., Hadala, J., Haston, W., Moreira-Ludewig, R., Uziel-Fusi, S., Peters, P., Bill, K., Wennogle, L.P., 1998. Novel C5a receptor antagonists regulate neutrophil functions in vitro and in vivo. J. Immunol. 160, 5616–5621.

Pemberton, M., Anderson, G., Vetvicka, V., Justus, D.E., Ross, G.D., 1993. Microvascular effects of complement blockade with soluble recombinant CR1 on ischemia/reperfusion injury of skeletal muscle. J. Immunol. 150, 5104–5113.

Picard, M.D., Pettey, C.L., Marsh, H.C., Thomas, L.J., 2000. Characterization of N-linked oligosaccharides bearing sialyl Lewis x moieties on an alternatively glycosylated form of soluble complement receptor type I (SCRI). Biotechnol. Appl. Biochem. 31, 5–13.

Piddlesden, S.J., Jiang, S.S., Levin, J.L., Vincent, A., Morgan, B.P., 1996. Soluble complement receptor 1 (sCR1) protects against experimental autoimmune myasthenia gravis. J. Neuroimmunol. 71, 173–177.

Piddlesden, S.J., Storch, M.K., Hibbs, M., Freeman, A.M., Lassmann, H., Morgan, B.P., 1994. Soluble recombinant complement receptor 1 inhibits inflammation and demyelination in antibody-mediated demyelinating experimental allergic encephalomyelitis. J. Immunol. 152, 5477–5484.

Pierre, A.F., Xavier, A.M., Liu, M.Y., Cassivi, S.D., Lindsay, T.F., Marsh, H.C., Slutsky, A.S., Keshavjee, S.H., 1998. Effect of complement inhibition with soluble complement receptor 1 on pig allotransplant lung function. Transplantation 66, 723–732.

Pratt, J.R., Hibbs, M.J., Laver, A.J., Smith, R.A.G., Sacks, S.H., 1996. Effects of complement inhibition with soluble complement receptor-1 on vascular injury and inflammation during renal allograft rejection in the rat. Am. J. Pathol. 149, 2055–2066.

Pruitt, S.K., Baldwin, W.M., Marsh, H.C.J., Lin, S.S., Yeh, C.G., Bollinger, R.R., 1991. The effect of soluble complement receptor type 1 on hyperacute xenograft rejection. Transplantation 52, 868–873.

Pruitt, S.K., Bollinger, R.R., 1991. The effect of soluble complement receptor type 1 on hyperacute allograft rejection. J. Surg. Res. 50, 350–355.

Pruitt, S.K., Kirk, A.D., Bollinger, R.R., Marsh, H.C., Collins, B.H., Levin, J.L., Mault, J.R., Heinle, J.S., Ibrahim, S., Rudolph, A.R., Baldwin, W.M., Sanfilippo, F., 1994. The effect of soluble complement receptor type 1 on hyperacute rejection of porcine xenografts. Transplantation 57, 363–370.

Quigg, R.J., 2002. Use of complement inhibitors in tissue injury. Trends Mol. Med. 8, 430–436.

Quigg, R.J., He, C., Hack, B.K., Alexander, J.J., Morgan, B.P., 2000. Production and functional analysis of rat CD59 and chimeric CD59-Crry as active soluble proteins in *Pichia pastoris*. Immunology 99, 46–53.

Quigg, R.J., He, C., Lim, A., Berthiaume, D., Alexander, J.J., Kraus, D., Holers, V.M., 1998a. Transgenic mice overexpressing the complement inhibitor Crry as a soluble protein are protected from antibody-induced glomerular injury. J. Exp. Med. 188, 1321–1331.

Quigg, R.J., Kozono, Y., Berthiaume, D., Lim, A., Salant, D.J., Weinfeld, A., Griffin, P., Kremmer, E., Holers, V.M., 1998b. Blockade of antibody-induced glomerulonephritis with Crry-Ig, a soluble murine complement inhibitor. J. Immunol. 160, 4553–4560.

Rabinovici, R., Yeh, C.G., Hillegass, L.M., Griswold, D.E., Dimartino, M.J., Vernick, J., Fong, K.L.L., Feuerstein, G., 1992. Role of complement in endotoxin/platelet-activating factor—induced lung injury. J. Immunol. 149, 1744–1750.

Regal, J.F., Fraser, D.G., Toth, C.A., 1993. Role of the complement system in antigen-induced bronchoconstriction and changes in blood pressure in the guinea pig. J. Pharmacol. Exp. Ther. 267, 979–988.

Rehrig, S., Fleming, S.D., Anderson, J., Guthridge, J.M., Rakstang, J., McQueen, C.E., Holers, V.M., Tsokos, G.C., Shea-Donohue, T., 2001. Complement inhibitor, complement receptor 1-related gene/protein y-Ig attenuates intestinal damage after the onset of mesenteric ischemia/reperfusion injury in mice. J. Immunol. 167, 5921–5927.

Riedemann, N.C., Guo, R.F., Laudes, I.J., Keller, K., Sarma, V.J., Padgaonkar, V., Zetoune, F.S., Ward, P.A., 2002. C5a receptor and thymocyte apoptosis in sepsis. FASEB J. 16, U378–U392.

Riley, R.D., Sato, H., Zhao, Z.Q., Thourani, V.H., Jordan, J.E., Fernandez, A.X., Ma, X.L., Hite, D.R., Rigel, D.E., Pellas, T.C., Peppard, J., Bill, K.A., Lappe, R.W., VintenJohansen, J., 2000. Recombinant human complement C5A receptor antagonist reduces infarct size after surgical revascularization. J. Thorac. Cardiovasc. Surg. 120, 350–358.

Rinder, C.S., Rinder, H.M., Smith, B.R., Fitch, J.C.K., Smith, M.J., Tracey, J.B., Matis, L.A., Squinto, S.P., Rollins, S.A., 1995. Blockade of C5a and C5b-9 generation inhibits leukocyte and platelet activation during extracorporeal circulation. J. Clin. Invest. 96, 1564–1572.

Rinder, C.S., Rinder, H.M., Smith, M.J., Tracey, J.B., Fitch, J., Li, L., Rollins, S.A., Smith, B.R., 1999. Selective blockade of membrane attack complex formation during simulated extracorporeal circulation inhibits platelet but not leukocyte activation. J. Thorac. Cardiovasc. Surg. 118, 460–466.

Rioux, P., 2001. TP-10 (AVANT immunotherapeutics). Curr. Opin. Investig. Drugs 2, 364–371.

Rittershaus, C.W., Thomas, L.J., Miller, D.P., Picard, M.D., Geoghegan-Barek, K.M., Scesney, S.M., Henry, L.D., Sen, A.C., Bertino, A.M., Hannig, G., Adari, H., Mealey, R.A., Gosselin, M.L., Couto, M., Hayman, E.G., Levin, J.L., Reinhold, V.N., Marsh, H.C., 1999. Recombinant glycoproteins that inhibit complement activation and also bind the selectin adhesion molecules. J. Biol. Chem. 274, 11237–11244.

Rollins, S.A., Matis, L.A., Springhorn, J.P., Setter, E., Wolff, D.W., 1995. Monoclonal antibodies directed against human C5 and C8 block complement-mediated damage of xenogeneic cells and organs. Transplantation 60, 1284–1292.

Roos, A., Nauta, A.J., Broers, D., FaberKrol, M.C., Trouw, L.A., Drijfhout, J.W., Daha, M.R., 2001. Specific inhibition of the classical complement pathway by C1q-binding peptides. J. Immunol. 167, 7052–7059.

Roos, A., Ramwadhdoebe, T.H., Nauta, A.J., Hack, C.E., Daha, M.R., 2002. Therapeutic inhibition of the early phase of complement activation. Immunobiology 205, 595–609.

Sahu, A., Kay, B.K., Lambris, J.D., 1996. Inhibition of human complement by a C3-binding peptide isolated from a phage-displayed random peptide library. J. Immunol. 157, 884–891.

Salerno, C.T., Kulick, D.M., Yeh, C.G., GuzmanPaz, M., Higgins, P.J., Benson, B.A., Park, S.J., Shumway, S.J., Bolman, R.M., Dalmasso, A.P., 2002. A soluble chimeric inhibitor of C3 and C5 convertases, complement activation blocker-2, prolongs graft survival in pig-to-rhesus monkey heart transplantation. Xenotransplantation 9, 125–134.

Scesney, S.M., Makrides, S.C., Gosselin, M.L., Ford, P.J., Andrews, B.M., Hayman, E.G., Marsh, H.C., 1996. A soluble deletion mutant of the human complement receptor type 1, which lacks the C4b binding site, is a selective inhibitor of the alternative complement pathway. Eur. J. Immunol. 26, 1729–1735.

Samsung Bioepis Exhibit No. 1002
Page 956

Schmid, E., Piccolo, M.T.S., Friedl, H.P., Warner, R.L., Mulligan, M.S., Hugli, T.E., Till, G.O., Ward, P.A., 1997. Requirement for C5a in lung vascular injury following thermal trauma to rat skin. Shock 8, 119–124.

Shandelya, S.M.L., Kuppusamy, P., Herskowitz, A., Weisfeldt, M.L., Zweier, J.L., 1993. Soluble complement receptor type 1 inhibits the complement pathway and prevents contractile failure in the post-ischemic heart evidence that complement activation is required for neutrophil-mediated reperfusion injury. Circulation 88, 2812–2826.

Sharma, A.K., Pangburn, M.K., 1994. Biologically active recombinant human complement factor H: synthesis and secretion by the baculovirus system. Gene 143, 301–302.

Shernan, S.K., Fitch, J.C., Nussmeier, N.A., Chen, J.C., Rollins, S.A., Mojcik, C.F., Malloy, K.J., Todaro, T.G., Filloon, T., Boyce, S.W., Gangahar, D.M., Goldberg, M., Saidman, L.J., Mangano, D.T., 2004. Impact of pexelizumab, an anti-C5 complement antibody, on total mortality and adverse cardiovascular outcomes in cardiac surgical patients undergoing cardiopulmonary bypass. Ann. Thorac. Surg. 77, 942–949.

Short, A., Wong, A.K., Finch, A.M., Haaima, G., Shiels, I.A., Fairlie, D.P., Taylor, S.M., 1999. Effects of a new C5a receptor antagonist on C5a- and endotoxin-induced neutropenia in the rat. Br. J. Pharmacol. 126, 551–554.

Smith, E.F., Griswold, D.E., Egan, J.W., Hillegass, L.M., Smith, R.A.G., Hibbs, M.J., Gagnon, R.C., 1993. Reduction of myocardial reperfusion injury with human soluble complement receptor type-1 (BRL-55730). Eur. J. Pharmacol. 236, 477–481.

Smith, G.P., Smith, R.A.G., 2001. Membrane-targeted complement inhibitors. Mol. Immunol. 38, 249–255.

Smith, R.A.G., 2002. Targeting anticomplement agents. Biochem. Soc. Trans. 30, 1037–1041.

Song, H., He, C., Knaak, C., Guthridge, J.M., Holers, V.M., Tomlinson, S., 2003. Complement receptor 2-mediated targeting of complement inhibitors to sites of complement activation. J. Clin. Invest. 111, 1875–1885.

Soulika, A.M., Khan, M.M., Hattori, T., Bowen, F.W., Richardson, B.A., Hack, C.E., Sahu, A., Edmunds Jr., L.H., Lambris, J.D., 2000. Inhibition of heparin/protamine complex-induced complement activation by compstatin in baboons. Clin. Immunol. 96, 212–221.

Sprong, T., Brandtzaeg, P., Fung, M., Pharo, A.M., Hoiby, E.A., Michaelsen, T.E., Aase, A., vanderMeer, J.W.M., VanDeuren, M., Mollnes, T.E., 2003. Inhibition of C5a-induced inflammation with preserved C5b-9-mediated bactericidal activity in a human whole blood model of meningococcal sepsis. Blood 102, 3702–3710.

Stahl, G.L., Xu, Y.Y., Hao, L.M., Miller, M., Buras, J.A., Fung, M., Zhao, H., 2003. Role for the alternative complement pathway in ischemia/reperfusion injury. Am. J. Pathol. 162, 449–455.

Stammberger, U., Hamacher, J., Hillinger, S., Schmid, R.A., 2000. sCR1sLe(X) ameliorates ischemia/reperfusion injury in experimental lung transplantation. J. Thorac. Cardiovasc. Surg. 120, 1078–1084.

Stevens, J.H., O Hanley, P., Shapiro, J.M., Mihm, F.G., Satoh, P.S., Collins, J.A., Raffin, T.A., 1986. Effects of anti-C5a antibodies on the adult respiratory distress syndrome in septic primates. J. Clin. Invest. 77, 1812–1816.

Strachan, A.J., Shiels, I.A., Reid, R.C., Fairlie, D.P., Taylor, S.M., 2001. Inhibition of immune-complex mediated dermal inflammation in rats following either oral or topical administration of a small molecule C5a receptor antagonist. Br. J. Pharmacol. 134, 1778–1786.

Strachan, A.J., Woodruff, T.M., Haaima, G., Fairlie, D.P., Taylor, S.M., 2000. A new small molecule C5a receptor antagonist inhibits the reverse-passive Arthus reaction and endotoxic shock in rats. J. Immunol. 164, 6560–6565.

Sugita, Y., Ito, K., Shiozuka, K., Suzuki, H., Gushima, H., Tomita, M., Masuho, Y., 1994. Recombinant soluble CD59 inhibits reactive haemolysis with complement. Immunology 82, 34–41.

Sumichika, H., Sakata, K., Sato, N., Takeshita, S., Ishibuchi, S., Nakamura, M., Kamahori, T., Ehara, S., Itoh, K., Ohtsuka, T., Ohbora, T., Mishina, T., Komatsu, H., Naka, Y., 2002. Identification of a potent and orally active non-peptide C5a receptor antagonist. J. Biol. Chem. 277, 49403–49407.

Szebeni, J. (Ed.), 2004. The Complement System: Novel Roles in Health and Disease. Kluwer Academic Publishers, Massachusetts.

Szebeni, J., Fontana, J.L., Wassef, N.M., Mongan, P.D., Morse, D.S., Dobbins, D.E., Stahl, G.L., Bunger, R., Alving, C.R., 1999. Hemodynamic changes induced by liposomes and liposome-encapsulated hemoglobin in pigs-A model for pseudoallergic cardiopulmonary reactions to liposomes: role of complement and inhibition by soluble CR1 and anti-C5a antibody. Circulation 99, 2302–2309.

Takahashi, K., Gordon, J., Liu, H., Sastry, K.N., Epstein, J.E., Motwani, M., Laursen, I., Thiel, S., Jensenius, J.C., Carroll, M., Ezekowitz, R.A., 2002. Lack of mannose-binding lectin-A enhances survival in a mouse model of acute septic peritonitis. Microbes Infect. 4, 773–784.

Tanhehco, E.J., Kilgore, K.S., Liff, D.A., Murphy, K.L., Fung, M.S., Sun, W.N., Sun, C., Lucchesi, B.R., 1999. The anti-factor D antibody, MAb 166-32, inhibits the alternative pathway of the human complement system. Transplant. Proc. 31, 2168–2171.

Theroux, P., Armstrong, P.W., Mahaffey, K.W., Hochman, J.S., Malloy, K.J., Rollins, S., Nicolau, J.C., Lavoie, J., Luong, T.M., Burchenal, J., Granger, C.B., 2005. Prognostic significance of blood markers of inflammation in patients with ST-segment elevation myocardial infarction undergoing primary angioplasty and effects of pexelizumab, a C5 inhibitor: a substudy of the COMMA trial. Eur. Heart J. (e-published ahead of print).

Thomas, T.C., Rollins, S.A., Rother, R.P., Giannoni, M.A., Hartman, S.L., Elliott, E.A., Nye, S.H., Matis, L.A., Squinto, S.P., Evans, M.J., 1996. Inhibition of complement activity by humanized anti-C5 antibody and single-chain Fv. Mol. Immunol. 33, 1389–1401.

Thurman, J.M., Kraus, D.M., Girardi, G., Hourcade, D., KANG, H.J., Royer, P.A., Mitchell, L.M., Giclas, P.C., Salmon, J., Gilkeson, G., Holers, V.M., 2005. A novel inhibitor of the alternative complement pathway prevents antiphospholipid antibody-induced pregnancy loss in mice. Mol. Immunol. 42, 87–97.

Tofukuji, M., Stahl, G.L., Agah, A., Metais, C., Simons, M., Sellke, F.W., 1998. Anti-C5a monoclonal antibody reduces cardiopulmonary bypass and cardioplegia-induced coronary endothelial dysfunction. J. Thorac. Cardiovasc. Surg. 116, 1060–1068.

Undar, A., Eichstaedt, H.C., Clubb, F.J., Fung, M., Lu, M.S., Bigley, J.E., Vaughn, W.K., Fraser, C.D., 2002. Novel anti-factor D monoclonal antibody inhibits complement and leukocyte activation in a baboon model of cardiopulmonary bypass. Ann. Thorac. Surg. 74, 355–362.

Vakeva, A.P., Agah, A., Rollins, S.A., Matis, L.A., Li, L., Stahl, G.L., 1998. Myocardial infarction and apoptosis after myocardial ischemia and reperfusion: role of the terminal complement components and inhibition by anti-C5 therapy. Circulation 97, 2259–2267.

Verrier, E.D., Shernan, S.K., Taylor, K.M., Van de Werf, F., Newman, M.F., Chen, J.C., Carrier, M., Haverich, A., Malloy, K.J., Adams, P.X., Todaro, T.G., Mojcik, C.F., Rollins, S.A., Levy, J.H., 2004. Terminal complement blockade with pexelizumab during coronary artery bypass graft surgery requiring cardiopulmonary bypass: a randomized trial. J. Am. Med. Assoc. 291, 2319–2327.

Wada, K., Montalto, M.C., Stahl, G.L., 2001. Inhibition of complement C5 reduces local and remote organ injury after intestinal ischemia/reperfusion in the rat. Gastroenterology 120, 126–133.

Walport, M.J., 2001a. Advances in immunology: complement (first of two parts). N. Engl. J. Med. 344, 1058–1066.

Walport, M.J., 2001b. Advances in immunology: complement (second of two parts). N. Engl. J. Med. 344, 1140–1144.

Wang, Y., Hu, Q.L., Madri, J.A., Rollins, S.A., Chodera, A., Matis, L.A., 1996. Amelioration of lupus-like autoimmune disease in NZB/WF1 mice after treatment with a blocking monoclonal antibody specific for complement component C5. Proc. Natl. Acad. Sci. U.S.A. 93, 8563–8568.

Wang, Y., Rollins, S.A., Madri, J.A., Matis, L.A., 1995. Anti-C5 monoclonal antibody therapy prevents collagen-induced arthritis and

Samsung Bioepis Exhibit No. 1002
Page 957

ameliorates established disease. Proc. Natl. Acad. Sci. U.S.A. 92, 8955–8959.

Weiler, J.M., Edens, R.E., Linhardt, R.J., Kapelanski, D.P., 1992. Heparin and modified heparin inhibit complement activation in vivo. J. Immunol. 148, 3210–3215.

Weiser, M.R., Pechet, T.T.V., Williams, J.P., Ma, M.H., Frenette, P.S., Moore, F.D., Kobzik, L., Hines, R.O., Wagner, D.D., Carroll, M.C., Hechtman, H.B., 1997. Experimental murine acid aspiration injury is mediated by neutrophils and the alternative complement pathway. J. Appl. Physiol. 83, 1090–1095.

Weisman, H.F., Bartow, T., Leppo, M.K., Marsh Jr., H.C., Carson, G.R., Concino, M.F., Boyle, M.P., Roux, K.H., Weisfeldt, M.L., Fearon, D.T., 1990. Soluble human complement receptor type 1: in vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. Science 249, 146–151.

Woodruff, T.M., Arumugam, T.V., Shiels, I.A., Reid, R.C., Fairlie, D.P., Taylor, S.M., 2003. A potent human C5a receptor antagonist protects against disease pathology in a rat model of inflammatory bowel disease. J. Immunol. 171, 5514–5520.

Woodruff, T.M., Strachan, A.J., Dryburgh, N., Shiels, I.A., Reid, R.C., Fairlie, D.P., Taylor, S.M., 2002. Antiarthritic activity of an orally active C5a receptor antagonist against antigen-induced monarticular arthritis in the rat. Arthritis Rheum. 46, 2476–2485.

Wurzner, R., Schulze, M., Happe, L., Franzke, A., Bieber, F.A., Oppermann, M., Gotze, O., 1991. Inhibition of terminal complement complex formation and cell lysis by monoclonal antibodies. Complement. Inflamm. 8, 328–340.

Xia, W., Fearon, D.T., Moore, F.D., Schoen, F.J., Ortiz, F., Kirkman, R.L., 1992. Prolongation of guinea pig cardiac xenograft survival in rats by soluble human complement receptor type-1. Transplant. Proc. 24, 479–480.

Yeh, C.G., Marsh Jr., H.C., Carson, G.R., Berman, L., Concino, M.F., Scesney, S.M., Kuestner, R.E., Skibbens, R., Donahue, K.A., Ip, S.H., 1991. Recombinant soluble human complement receptor type 1 inhibits inflammation in the reversed passive Arthus reaction in rats. J. Immunol. 146, 250–256.

Yoshitatsu, M., Miyagawa, S., Mikata, S., Matsunami, K., Yamada, M., Murase, A., Sawa, Y., Ohtake, S., Matsuda, H., Shirakura, R., 1999. Function of human factor H and I on xenosurface. Biochem. Biophys. Res. Commun. 265, 556–562.

Zacharowski, K., Otto, M., Hafner, G., Marsh, H.C., Thiemermann, C., 1999. Reduction of myocardial infarct size with sCR1sLe(X), an alternatively glycosylated form of human soluble complement receptor type 1 (SCR1), possessing sialyl Lewis x. Br. J. Pharmacol. 128, 945–952.

Zhang, H.F., Lu, S.L., Morrison, S.L., Tomlinson, S., 2001. Targeting the functional antibody-decay-accelerating factor fusion proteins to a cell surface. J. Biol. Chem. 276, 27290–27295.

Zhang, H.F., Yu, J.H., Bajwa, E., Morrison, S.L., Tomlinson, S., 1999. Targeting of functional antibody-CD59 fusion proteins to a cell surface. J. Clin. Invest. 103, 55–61.

Zhao, H., Wakamiya, N., Suzuki, Y., Hamonko, M.T., Stahl, G.L., 2002. Identification of human mannose binding lectin (MBL) recognition sites for novel inhibitory antibodies. Hybrid. Hybridomics 21, 25–36.

Zimmerman, J.L., Dellinger, R.P., Straube, R.C., Levin, J.L., 2000. Phase I trial of the recombinant soluble complement receptor 1 in acute lung injury and acute respiratory distress syndrome. Crit. Care Med. 28, 3149–3154.

Samsung Bioepis Exhibit No. 1002
Page 958

ALEXION OFFERS EARLY, MIXEDPHASE III DATA FOR HEART DRUG

Author: Osborne, Randall

Publication info: BIOWORLD Today 14.151 (Aug 6, 2003): NA.

ProQuest document link

Abstract (English): Byline: Randall Osborne , National Editor

Alexion Pharmaceuticals Inc. narrowly missed its primary endpoint in the preliminary analysis of its Phase III heart surgery drug, pexelizumab, but the company said other indicators are encouraging enough to ask the FDA about filing for approval.

But that didn't thrill Wall Street, which took away 21.8 percent from Alexion shares. Its stock (NASDAQ:ALXN) fell $3.49 Tuesday to close at $12.51, after dipping as low as $12.03.

Leonard Bell, CEO of Cheshire, Conn.-based Alexion, declined to estimate when the company will know its next step with the drug, but told BioWorld Today, "What we need to do is complete all of our internal diligence" and begin talks with the FDA.

Full text: Byline: Randall Osborne , National Editor

Alexion Pharmaceuticals Inc. narrowly missed its primary endpoint in the preliminary analysis of its Phase III heart surgery drug, pexelizumab, but the company said other indicators are encouraging enough to ask the FDA about filing for approval.

But that didn't thrill Wall Street, which took away 21.8 percent from Alexion shares. Its stock (NASDAQ:ALXN) fell $3.49 Tuesday to close at $12.51, after dipping as low as $12.03.

Leonard Bell, CEO of Cheshire, Conn.-based Alexion, declined to estimate when the company will know its next step with the drug, but told BioWorld Today, "What we need to do is complete all of our internal diligence" and begin talks with the FDA.

Full data from the 3,000-patient trial, called "Pexelizumab for Reduction in Infarction and Mortality in Coronary Artery Bypass Graft Surgery," or the PRIMO-CABG trial, won't be disclosed until November at the annual meeting of the American Heart Association.

Embargo rules prevent Alexion from disclosing more about the Phase III results now, Bell said, but noted in a conference call there was "substantial internal consistency in the data set. We think that the sum total of the data of pexelizumab in CABG is supportive of having a clinically meaningful effect."

In the figures that could be disclosed, Alexion's short-acting, single- chain antibody C5 complement inhibitor failed to hit its primary endpoint: reducing death and myocardial infarction at 30 days in patients undergoing CABG who were not undergoing valve surgery at the same time.

Between 10 percent and 12 percent of CABG patients undergo valve surgery at the same time as their bypass graft procedure, but Alexion disqualified them from primary analysis of the trial since Phase II data suggested pexelizumab is less likely to work in that setting.

In the Phase III study, however, the drug did reach borderline statistical significance ($p<0.05$) for both sets of patients - those who underwent valve surgery and those who didn't - in reducing death and myocardial infarction. That "all comers" analysis made up one of the secondary endpoints.

Alexion will provide no further p values or scientific details until the
AHA meeting.

Eric Schmidt, analyst with SG Cowen Securities Corp., called the secondary endpoint finding "ironic" and meaningful in a favorable way. Bell agreed the Phase III outcome is ironic but said the choice to exclude the valve surgery patients from the primary analysis was the right one at the time.

"Based on the data we had from the Phase II with only 30 patients in the group, I don't think we were in a position to judge whether it had a discernible effect in that population," he said.

Although Schmidt acknowledged that approval of the drug based on PRIMO-CABG data is "far from assured," a biologics license application could be filed as early as 2004, and the revenue potential in the U.S. is between $300 million and $500 million. Alexion's clinical advisers believe the company will "receive a green light to at least file for approval," Schmidt wrote in a research note.

Bell was circumspect, but said the worst-case scenario would be that the FDA decides "the data are in no way supportive, in which case you ought to stop. That's a low likelihood."

Pexelizumab is partnered with Procter &Gamble Pharmaceuticals, an affiliate of Procter &Gamble Co., of Cincinnati. Alexion shares equally in development and commercialization, handling about half of sales efforts and taking half the profits. (See BioWorld Today, Dec. 13, 2001.)

Other secondary endpoints in the Phase III trial include psychomotor function, the effect of pexelizumab on death alone and the drug's effect on perioperative (that is, from the time of hospitalization for surgery to the time of discharge) Q-wave and non-Q-wave myocardial infarction. Details on those endpoints are expected at the AHA meeting to be held in Orlando, Fla.

Meanwhile, the company offered only an outline of the Phase III data, with an opinion of what they might mean. "We believe we have hit a solid triple, and we're now focusing on the best strategy to drive the drug home," Bell said during a conference call, pointing to a significant unmet medical need.

"There are no approved drugs that we are familiar with that limit death or heart attack in this patient population or have been shown to do so," he said.

Behind pexelizumab in the Alexion pipeline is eculizumab, formerly known as 5G1.1, a fully humanized whole antibody (as opposed to single chain), which in Phase I/II and Phase II trials has shown proof- of-principle efficacy in rheumatoid arthritis, lupus/membranous nephritis and dermatomyositis.

Alexion has a manufacturing deal with Lonza Biologics Ltd., of Basel, Switzerland, for eculizumab, which Schmidt said has yielded the most promising data in a Phase I/II study in paroxysmal nocturnal hemoglobinuria, which is associated with a high risk of thrombotic events.

"We would expect over the next several months we would be able to announce our plans [regarding eculizumab]," Bell said.

Subject: Cardiovascular agents -- Product development;Company securities;Pharmaceutical industry -- Product development;Pharmaceutical industry -- Securities;Product development;Securities

Location: United States

Company / organization: Alexion Pharmaceuticals Inc.; Alexion Pharmaceuticals Inc. (Product development); Alexion Pharmaceuticals Inc. (Securities)

Identifier (keyword): Trade, ALXN

Event classification: 331: Product development;810: Securities issued, listed

NAICS classification: 325412: Pharmaceutical Preparation Manufacturing

Product classification: 2834300: Cardiovascular Preparations

SIC classification: 2834: Pharmaceutical preparations

Publication title: BIOWORLD Today

Volume: 14

Issue: 151

Pagination: NA

Publication date: Aug 6, 2003

Publisher: AHC Media LLC

Journal subject: Biotechnology industry, Business

ISSN: 1541-0595

Journal code: 0EUH

Source type: Magazine/Journal

Language of publication: English

Document type: Magazine/Journal

Source attribution: Gale Group Newsletter Database, © Publisher specific

Accession number: 106306086

Document URL: http://dialog.proquest.com/professional/docview/10754218342?accountid=157282

Copyright: Copyright 2003 Gale, Cengage Learning. All rights reserved.COPYRIGHT 2003 A Thomson Healthcare Company

Date created: 2012-01-27

Updates: 2012-09-26

First available: 2012-09-26

Database: Gale Group Newsletter Database™ (1988 - current)

Contact ProQuest

Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

Current methods for the generation of human antibodies for the treatment of autoimmune diseases

Jane Osbourn, Lutz Jermutus and Alex Duncan

Autoimmune diseases are a significant area of unmet medical need in the Western World, but human antibodies are an emerging drug class that could address this demand. Some autoimmune diseases, such as rheumatoid arthritis, are currently benefiting from antibody treatment and new and existing technologies for antibody generation could facilitate the production of effective human antibodies as future drug candidates for other autoimmune diseases. Several methods of generating human antibodies for use as therapeutics have been established, the most commonly used being phage display and transgenic mouse technologies and more recently, cell-free display technologies have also emerged. In this review, we explain the principles behind the various methods of antibody generation and highlight some potential benefits of certain approaches in the context of treatment of autoimmune disease.

Jane Osbourn*
Lutz Jermutus
Alex Duncan
Cambridge Antibody Technology, Milstein Building Granta Park, Cambridge UK CB1 6GH
*e-mail: jane.osbourn@cambridgeantibody.com

The American Autoimmune Related Diseases Association (www.aarda.org) estimates that there are >80 autoimmune diseases, which, collectively, after heart disease and cancer, cause one third of all premature deaths and disability in the Western World. Among the most prevalent are Graves' Disease, rheumatoid arthritis (RA), Hashimoto's thyroiditis, multiple sclerosis (MS), Raynaud's syndrome, systemic lupus erythematosus (SLE), Sjogren's syndrome, scleroderma and Crohn's disease [1].

Disease mechanisms

The normal function of the immune system is to respond to invading microorganisms by producing antibodies or sensitized lymphocytes to recognize and destroy them. Autoimmune diseases occur when the normal function of the immune system is disrupted and antibodies to the patient's own cells and tissues are produced. These antibodies can interfere with cellular function and initiate tissue destruction, producing inflammation in the surrounding tissue or organ. The disease-producing processes in autoimmunity are referred to as hypersensitive reactions, which are similar to those observed in allergy, except that in autoimmune disorders, the hypersensitivity response is to the body itself, rather than to an outside substance.

Autoimmune disorders are classified into organ-specific and non-organ-specific types. In organ-specific types, the reaction is directed against one particular organ – examples include Hashimoto's thyroiditis (thyroid) and Addison's disease (adrenal glands). In non-organ-specific disorders, the effects are widespread throughout the body, for example SLE and RA. Most autoimmune diseases are chronic, requiring lifelong care and monitoring, and no cure for any of these diseases has yet been developed.

Current treatments

In autoimmune disorders, such as RA, established treatments relieve pain and inflammation, or suppress the immune response. Pain-relief drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids reduce inflammation but are also associated with a wide range of side-effects. Commonly used NSAIDs, such as the cyclooxygenase 2 (COX-2) inhibitors, can cause a variety of problems, including gastrointestinal toxicity, which results in abdominal pain, nausea, indigestion and ulcers [2,3]. Corticosteroids, such as prednisone, are highly effective at relieving pain but can cause weight gain [4] and, in some patients, can contribute to the development of osteoporosis [5].

1359-6446/03/$ – see front matter ©2003 Elsevier Science Ltd. All rights reserved. PII: S1359-6446(03)02803-4

Samsung Bioepis Exhibit No. 1002
Page 962

Alternative RA treatments, aimed at slowing joint destruction, include a range of widely used disease-modifying anti-rheumatic drugs (DMARDs), such as methotrexate and chlorquine. Although effective, these drugs must be taken for several months before they show any positive effects. They are also non-specific to the site of inflammation and therefore, cause side effects ranging from abdominal discomfort, rashes, gastrointestinal toxicity and nausea to decreases in platelets, or white and red blood cell counts [6].

The variety of severe side effects associated with RA treatments has prompted a move towards the development of drugs that directly target the causes of the disease rather than attempting to alleviate the symptoms. For RA treatment, these 'biological response modifiers' (BRMs) directly target the cytokines, such as tumour necrosis factor (TNF-α), that are involved in the development and progression of the disease. Treatments include Enbrel®, a TNF-α receptor protein [7], and the antibody drugs Remicade® [8] and HUMIRA™ [9], which bind specifically to TNF-α. In clinical trials, these BRMs have demonstrated efficacy in reducing the symptoms of RA and in inhibiting joint erosion. Antibodies are particularly suited to the role of a BRM, because they can bind specifically and with high affinity to a wide range of biological molecules and, because they have a longer half-life than small-molecule treatments.

Monoclonal antibodies

The production of hybridoma cell lines is perhaps the most established technique for generating monoclonal antibodies [10]. Hybridomas are generated by immunizing a mouse with a target antigen, then fusing the antibody-producing B cells of that mouse with mouse tumour cells. The hybridoma possesses the immortal growth properties of the tumour cell and thus, can be cultured indefinitely, and can secrete antibody with the specificity of the original B cell. The homogenicity and specificity of monoclonal antibodies that are produced from hybridomas makes them particularly suitable for *in vivo* administration for therapeutic purposes. However, clinical studies using mouse monoclonal antibodies have been disappointing, because the human immune system recognizes the mouse antibodies as 'foreign'. This results in rapid clearance of the mouse protein by the immune system of the patient and, in some cases, can lead to the induction of a severe allergic reaction to the mouse antibodies – an effect known as the human anti-mouse antibody (HAMA) response.

Chimaeric monoclonal antibodies

To reduce the potential for HAMA responses, variable regions from mouse antibody genes can be recombined with constant regions from human antibody genes. The recombinant gene encodes a chimaeric mouse–human antibody that has antigenic specificity derived from the mouse, but a human isotype. Hence, the resultant protein has human effector functions and fewer mouse antigenic determinants, and is therefore less likely to be immunogenic in humans. Mouse variable regions can also cause an immune response in humans, and chimaeric antibodies containing only mouse complementarity determining regions (CDRs) have subsequently been developed. CDRs are the regions of the antibody molecule that are largely responsible for antibody–antigen binding and including only these elements rather than the full variable region of the antibody, further reduces the risk of immunogenicity. Replacing some mouse CDR sequences with human sequences can further humanize antibodies; mouse residues are minimized but retained at the key binding regions, to maintain affinity.

By the start of 2003, nine monoclonal antibodies had been granted a biologics licence approval (BLA) by the US Food and Drug Administration (FDA) (www.fda.gov) and all of these were of the chimaeric mouse–human or humanized type. These antibodies are being successfully used to treat a variety of conditions, including leukaemia, organ rejection, non-Hodgkin's lymphoma and breast cancer. In addition, one of these chimaeric antibodies, Remicade®, is approved for the treatment of the autoimmune disorders, RA and Crohn's disease, and there are also several other chimaeric or humanized antibodies currently in clinical trials for the treatment of a range of autoimmune diseases (Table 1).

Many chimaeric antibodies still cause adverse events, such as headache, fever, chills [11] and respiratory infection [12] and, in some patients, these events can be associated with allergic reactions to the drug. One solution to some of the immunogenic responses induced by chimaeric antibody drugs is to develop human antibody therapeutics, which contain no mouse-derived sequences. Transgenic mice and phage display techniques are the key technologies that have been widely used to isolate such human antibodies.

Transgenic mice

Transgenic mouse technology for antibody drug discovery involves the introduction of human antibody genes into the mouse genome [13,14]. The mice are engineered to suppress their own antibody production and large sections of the human antibody heavy and light chain loci are then transferred into these animals; the human antibody genes are functional in the context of the mouse machinery for antibody recombination and expression. The mouse antibody-

Samsung Bioepis Exhibit No. 1002
Page 963

Table 1. Chimaeric and humanized antibodies developed for the treatment of autoimmune diseases

Product name	Generic name	Antibody type	Indication	Clinical development phase	Company
Remicade®	Infliximab	Anti-TNF-α	Crohn's Disease, RA, psoriasis	FDA approved	Centocor
Campath®	Alemtuzumab	Anti-CD52	MS	FDA approved	BTG, ILEX, Millennium, Schering AG
Raptiva™	Efalizumab	Anti-CD11	Psoriasis	Phase III BLA filed	Xoma, Serono, Genentech
Antegren®	Natalizumab	Anti-α_4 integrin	MS	Phase III	Elan, Biogen, Genzyme
Humicade™	CDP 571	Anti-TNF-α	Crohn's Disease	Phase III	Celltech, Biogen, Genzyme
CDP 870	CDP 870	Anti-TNF-α	RA and Crohn's Disease	Phase III	Celltech, Pharmacia
Antegren®	Natalizumab	Anti-α_4 integrin	MS, Crohn's Disease	Phase III	Elan, Biogen, Genzyme
MLN02	MLN02	Anti-$\alpha_4\beta_7$ integrin	Crohn's Disease	Phase II	Millennium, Genentech
HuZAF	Fontolizumab	Anti-interferon-γ	Crohn's Disease, psoriasis	Phase II	PDL
IDEC-151	Clenoliximab	Anti-CD4	RA	Phase II	IDEC
Zenapax®	Daclizumab	Anti-IL-2	Uveitis	Phase II	PDL
5G1.1	Eculizumab	Anti-C5	RA	Phase II	Alexion
MEDI-507	Siplizumab	CD2 agonist	Psoriasis	Phase II	Biotransplant, MedImmune

Abbreviations: IL, interleukin; MS, multiple sclerosis; RA, rheumatoid arthritis; TNF, tumour necrosis factor.

producing B cells can then be used to generate hybridoma cell lines for human monoclonal antibody production. In the usual time span required for hybridoma generation and characterization, a transgenic mouse can produce a range of specific antibodies from which potential drug candidates can be identified. If further optimization of the antibody is required, the genomic information that encodes the antibody of interest must be cloned and alternative antibody engineering approaches employed.

Companies using this approach include Abgenix (http://www.abgenix.com) and Medarex (http://www.medarex.com). Medarex has licensed its technology to Genmab A/S (http://www.genmab.com) and also collaborates with Kirin Brewery (http://www.kirin.co.jp/). These companies have taken antibody drugs into various stages of clinical trials, with Medarex having two antibodies to treat different autoimmune diseases in mid-stage clinical trials (Table 2). No significant allergic reactions have been noted so far and thus, the safety profiles seem better than with chimaeric antibodies.

Phage display

The other established method of developing human monoclonal antibodies is phage display technology. This

Table 2. Human antibodies in development to treat autoimmune diseases

Product name	Generic name	Antibody type	Indication	Method of generation	Clinical development phase	Company
HUMIRA™	Adalimumab	Anti-TNF-α	RA	Phage display	FDA approved	CAT, Abbott
HuMax-CD4		Anti-CD4	Psoriasis	Transgenic mice	Phase II	Genmab, EISAI
HuMax-IL15		Anti-IL-15	RA, psoriasis	Transgenic mice	Phase II	Genmab, Medarex
HUMIRA™	Adalimumab	Anti-TNF-α	Juvenile RA, Crohn's Disease	Phage display	Phase II	CAT, Abbott
CAT-192	Metelimumab	Anti-TGF-β	Scleroderma	Phage display	Phase II	CAT, Genzyme
ABT-874		Anti-IL-12	Crohn's disease, RA	Phage display	Phase II	CAT, Abbott, Wyeth Research

Abbreviations: IL, interleukin; MS, multiple sclerosis; RA, rheumatoid arthritis; TGF, tissue growth factor.

Samsung Bioepis Exhibit No. 1002
Page 964

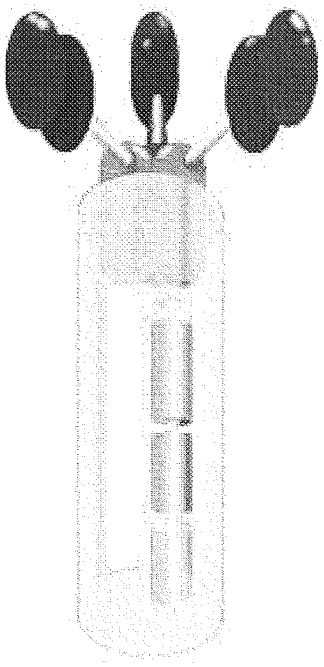


Figure 1. A filamentous phage displaying an antibody protein at its tip. An antibody variable region sequence is inserted into the phage genome linked to the gene 3 coat-protein sequence. The antibody sequence is then 'displayed' as a gene 3 fusion protein on the outside of the phage. The key feature of phage display is that the phenotype and genotype are linked in a single molecule.

technique, as first described for antibodies in 1990 [15], exploits the ability of filamentous bacteriophage, such as M13, to express foreign proteins on the phage surface (Figure 1), enabling the gene and gene product to be physically linked.

Phage antibody libraries are produced by cloning variable regions of human antibody genes, derived from naïve, immunized or synthetic antibody repertoires, into the phage coat-protein genes of phagemid vectors. These libraries are introduced into *Escherichia coli* cells, which are then superinfected with helper phage, to facilitate the replication and assembly of viable phage particles. Phage libraries contain a huge variety of antibody variable region sequences [16] and some libraries now contain $>10^{11}$ unique phage antibodies. Each phage particle in the library encapsidates the DNA that encodes the antibody expressed on its surface and antibodies specific to a defined target can be selected from these large phage libraries by allowing the library to bind to immobilized antigen – phage antibodies that bind to the antigen are thereby captured and non-binding phage washed away. Antibody variable regions selected using this technique can be reformatted into IgG molecules by combination with human antibody constant regions and subsequently expressed in mammalian cell lines.

The great advantage of phage display is that once a library has been created, it can be used to select for antibodies that bind to any target antigen of interest, with the selection and primary screening process being completed in only 2–3 weeks.

Selection from the library can also be tailored to produce a range of output populations, depending on the desired characteristics of the antibody drug. For example, it might be necessary to generate an antibody with the ability to bind to a specific epitope on the target molecule, the ability to cross-react with a rodent antigen, or the ability to recognize a given cell type to name but a few. When an antibody with the desired characteristics has been isolated, the phage system also provides the format for the engineering of antibody affinity or potency. The diversity of antibody populations generated using phage display means that antibodies that bind to different parts of the same target antigen molecule can be isolated. This also makes the technique highly applicable to functional genomics, because it can rapidly generate lead candidate antibodies to large numbers of proteins in high throughput.

Several biotechnology companies use phage display technology to produce human antibody therapeutics, including Cambridge Antibody Technology (CAT, http://www.cambridgeantibody.com), Crucell (http://www.crucell.com), Dyax (http://www.dyax.com) and MorphoSys (http://www.morphosys.de/). The world's first human antibody to treat RA, HUMIRA™, is now on the market. This antibody was isolated and optimized by CAT in collaboration with Abbott Laboratories (http://abbott.com). All the human antibodies that are currently in clinical trials (from Phase II) for autoimmune diseases are listed in Table 2.

HUMIRA™ demonstrates the effectiveness of human monoclonal antibodies derived from phage display. Published data from a study of 271 patients showed that HUMIRA™ improved the symptoms of RA in up to 50% of patients. In addition, only minor side effects, such as injection-site reactions, rashes and headaches were reported, suggesting that the drug is safe and well tolerated at the doses used [9].

Abbott submitted information from this trial and several others to the FDA and European Agency for the Evaluation of Medicinal Products (EMEA, http://www.emea.eu.int/), obtaining marketing approval from the FDA on 31 December 2002. This made HUMIRA™ the first human monoclonal antibody therapy on the market for the treatment of an autoimmune disease.

Samsung Bioepis Exhibit No. 1002
Page 965

Emerging *in vitro* display technologies for creating human antibodies

Transgenic mice and phage display are both cell-based development technologies, and hence, they are limited by the physical constraints of getting DNA into a cellular host. To overcome this restriction, two new cell-free translation technologies have been developed, known as 'covalent display' and 'ribosome display'. These techniques promise the rapid construction of display libraries, containing up to 10^{14} molecules – significantly larger than phage display libraries – and thus, provide an increased probability that the desired specificity and potency required in the therapeutic candidate can be directly selected.

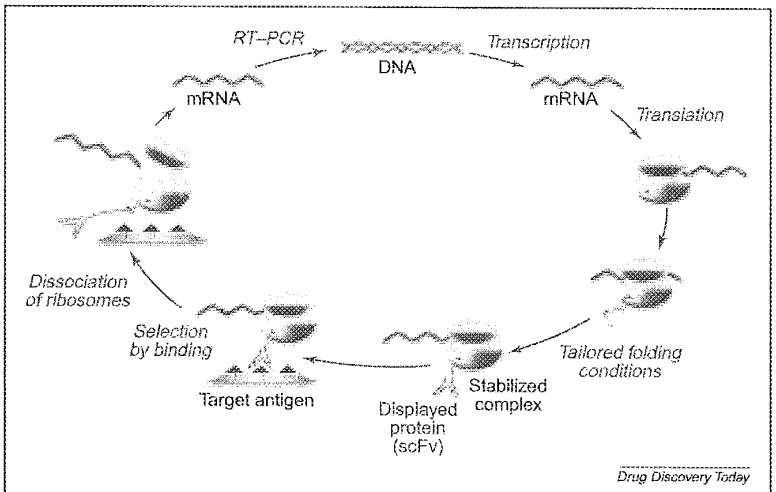


Figure 2. The ribosome display process. This cell-free process, analogous to phage display, involves the following steps: i) *in vitro* transcription of an antibody DNA library; ii) *in vitro* translation of mRNA molecules that contain a short spacer sequence and lack a stop codon; iii) folding of antibody molecule while tethered to the ribosome and encoding mRNA; iv) selection of antibody–ribosome–mRNA complexes by binding to immobilized target antigen (any non-binding antibodies are washed away); v) release of selected mRNA molecules by alteration of wash conditions and; vi) generation of DNA by RT–PCR for use in further rounds of selection.

Ribosome display

Ribosome display works by exploiting the cellular role of ribosomes to synthesize proteins. Ribosomes do this by binding to mRNA and travelling along the molecule, reading the nucleotide sequence and assembling a chain of amino acids as they move. In the cell, the fully formed protein is then released and the mRNA dissociates from the ribosome. The ribosome display system uses mRNA encoding a repertoire of antibody genes in a cell-free expression system (either *E. coli* or eukaryotic cell lysate), so enabling assembly of antibody molecules *in vitro*.

A short mRNA spacer is included at the beginning of the antibody sequence to ensure that the antibody molecule is fully extruded from the ribosomal tunnel. Cellular mRNA molecules have a start and stop codon on either side of the protein coding sequence; in the ribosome display system, the stop codon at the end of the antibody-coding region is removed, preventing the release of the newly translated antibody protein. The antibody remains tethered to the ribosome, which, in turn, remains associated with the corresponding antibody gene in the form of mRNA (Figure 2). Conditions in the cell-free lysate environment can be tailored to ensure the correct folding of the antibody molecule.

The ribosome display selection process is analogous to that of phage display, with antibody–ribosome– mRNA complexes being selected by binding to immobilized target antigen and any non-binding antibodies being washed away. The selected mRNA can be released from the ribosomes by alteration of the salt concentration of the wash buffer. RT–PCR can then be performed to generate a population of selected DNA that can be used as the starting point for further rounds of selection

Using ribosome display, single-chain antibodies specific for a yeast transcription factor GCN4 have been selected [17]. In addition, ribosome display has been successfully used to generate anti-progesterone antibody fragments [18]. There is also evidence that ribosome display can produce variable domain (CTLA-4) libraries and could be used to provide specific targeting to either novel or refractory cancer markers [19]. Ribosome display technology (acquired and developed for commercial purposes by CAT) has the potential to become a versatile tool for the development of human monoclonal antibodies [20] with picomolar affinities [21].

The main advantage of this technique is that it has the potential to generate larger libraries of antibodies than phage display, hence producing still more diverse selectable populations, while offering many of the same benefits of a phage display library. These include the ability to produce and screen the library for binding activities in a matter of weeks and the provision of a format for the directed evolution of antibodies to improve desirable characteristics, such as affinity, stability and potency [22].

Samsung Bioepis Exhibit No. 1002
Page 966

Covalent display

Other emerging display technologies, known as covalent display, have been developed by Isogenica [23] (http:// www.isogenica.com) and Phylos [24] (http://www.phylos.com).

The Phylos PROfusion™ mRNA display system relies upon puromycin-mediated covalent coupling of protein to the RNA that encodes it [25], and is conducted entirely as isolated biochemical reactions, outside of the host cells. The process begins with oligonucleotide synthesis to construct a population of DNA molecules, each containing defined regions with signals for RNA transcription, protein translation, purification tags and PCR amplification. The molecules also have regions of diversity, which encode for a protein scaffold and a unique protein-binding site [26].

The library DNA is transcribed to generate RNA and a peptide acceptor is covalently attached to the 3′ end of mRNA, using a synthetic linker. During *in vitro* translation in a cellular lysate, the ribosome moves along the mRNA to generate a protein.

When the ribosome has reached the end of the encoding mRNA, it pauses, allowing time for the peptide acceptor to covalently link to the C-terminus of the protein. The result is a collection of up to 10^{13} possible binding proteins, each chemically linked to the RNA that encodes it.

The molecules can then be purified from the reaction mixture using target proteins that are captured on magnetic beads. The beads are washed to remove nonspecific or weakly binding molecules and the nucleic acid of the PROfusion™ molecules that bind to the target molecule are recovered by PCR, creating a new pool of nucleic acids enriched with high-affinity binders for the target of interest.

The Isogenica system uses a replication initiator protein (P2A) from the *E. coli* phage, P2, to initiate rolling circle replication by binding to the viral origin and introducing a nick in the DNA. This exposes the 3′-OH group, which then initiates DNA synthesis [27–29]. Nicking also exposes a 5′ phosphate, which attaches to a tyrosine residue in the active site of P2A, thus binding the P2A to the DNA molecule from which it has been expressed. This enables pools of polypeptides that are fused to P2A to be synthesized *in vitro*, so that they become attached to their own coding sequence and can be selected on an immobilized antigen.

Recently, the Phylos PROfusion™ mRNA display system has been used to produce protein microarrays [30]. However, neither of the technologies described has, to date, generated a therapeutic antibody, although development is ongoing.

Summary and prospects

One chimaeric monoclonal antibody (Remicade®) has been FDA-approved as a treatment for RA and Crohn's disease and 11 are in mid–late-stage clinical trials; to date, chimaeric or humanized antibodies are the most commercially advanced types of antibody therapeutics for the treatment of autoimmune diseases. This is due partly to the fact that methods for generating chimaeric antibodies have been available for longer than the alternative technologies and have, therefore, produced more antibodies for clinical development. Many of these antibodies have significant side effects relating to their immunogenicity in human patients.

With the approval of HUMIRA™ (the first human antibody to treat RA) and an additional four human antibodies in Phase II or III trials for the treatment of autoimmune diseases, transgenic mice and phage display systems are now proving effective in the field of drug development. The benefit of these technologies is the ability to generate wholly human antibodies, which do not produce the immunogenic side effects that are seen with infusions of chimaeric antibodies. Short lead-generation times for antibodies made by phage display or transgenic mouse technology and, in the case of phage display, the ability to further optimize these lead candidates, makes the possibility of high potency therapeutics from these methods a reality.

Owing to their relatively recent emergence, neither covalent nor ribosome display has so far produced any human antibodies for use as clinical candidates in autoimmune disease. However, both technologies can produce libraries that are potentially larger than either phage display or chemical libraries, and so they could, in the next five years, become more widely adopted. Successful technical developments already demonstrated within the ribosome display system could mean that these technologies become the systems of choice for the generation and optimization of human antibodies against new drug targets for autoimmune diseases in the near future.

References

1 Jacobson, D.L. et al. (1997) Epidemiology and estimated population burden of selected autoimmune diseases in the United States. *Clin. Immunol. Immunopathol.* 84, 223–243

2 Bombardier, C. (2002) An evidence-based evaluation of the gastrointestinal safety of coxibs. *Am. J. Cardiol.* 89, 3D–9D

3 Laine, L. (2003) Gastrointestinal effects of NSAIDs and coxibs. *J. Pain Symptom Manage.* 25(Suppl. 2), S32–S40

4 Conn, D.L. and Lim, S.S. (2003) New role for an old friend: prednisone is a disease-modifying agent in early rheumatoid arthritis. *Curr. Opin. Rheumatol.* 15, 193–196

5 Emkey, R. et al. (2003) Changes in bone mineral density following discontinuation or continuation of alendronate therapy in glucocorticoid-treated patients: a retrospective, observational study. *Arthritis Rheum.* 48, 1102–1108

6 Blomqvist, P. et al. (2000) Rheumatoid arthritis in Sweden. Drug prescriptions, costs, and adverse drug reactions. *J. Rheumatol.* 27, 1171–1177

7 Bathon, J.M. et al. (2000) A comparison of etercept and methotrexate in patients with early rheumatoid arthritis. *N. Engl. J. Med.* 342, 1586–1593

Samsung Bioepis Exhibit No. 1002
Page 967

8 Lipsky, P.E. et al. (2000) Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-tumour necrosis factor trial in rheumatoid arthritis with concomitant therapy study group. *N. Engl. J. Med.* 343, 1594–1602

9 Weinblatt, M.E. et al. (2003) Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. *Arthritis Rheum.* 48, 35–45

10 Köhler, G. and Milstein, C. (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256, 495–497

11 Schaible, T.F. (2001) Treatment of inflammatory diseases: safety of long-term use of infliximab. *Presse Med.* 30, 610–613

12 Van Den Bosch, F. et al. (2002) Randomized double-blind comparison of chimeric monoclonal antibody to tumor necrosis factor alpha (infliximab) versus placebo in active spondylarthropathy. *Arthritis Rheum.* 46, 755–765

13 Green, L.L. et al. (1994) Antigen-specific human monoclonal antibodies from mice engineered with human Ig heavy and light chain YACs. *Nat. Genet.* 7, 13–21

14 Mendez, M.J. et al. (1997) Functional transplant of megabase human immunoglobulin loci recapitulates human antibody response in mice. *Nat. Genet.* 15, 146–156

15 McCafferty, J. et al. (1990) Phage antibodies: filamentous phage displaying antibody variable domains. *Nature* 348, 552–554

16 Vaughan, T. et al. (1996) Human antibodies with sub-nanomolar affinities isolated from large non-immunised phage display library. *Nat. Biotechnol.* 14, 309–314

17 Hanes, J. and Plückthun, A. (1997) In vitro selection and evolution of functional proteins by using ribosome display. *Proc. Natl. Acad. Sci. U. S. A.* 94, 4937–4942

18 He, M. et al. (1999) Selection of a human anti-progesterone antibody fragment from a transgenic mouse library by ARM ribosome display. *J. Immunol. Methods* 231, 105–117

19 Irving, R.A. et al. (2000) Ribosome display and affinity maturation: from antibodies to single V-domains and steps towards cancer therapeutics. *J. Immunol. Methods* 248, 31–45

20 Hanes, J. et al. (1998) Ribosome display efficiently selects and evolves high-affinity antibodies in vitro from immune libraries. *Proc. Natl. Acad. Sci. U. S. A.* 95, 14130–14135

21 Hanes, J. et al. (2000) Picomolar affinity antibodies from a fully synthetic naive library selected and evolved by ribosome display. *Nat. Biotechnol.* 18, 1287–1292

22 Jermutus, L. et al. (2001) Tailoring in vitro evolution for protein affinity or stability. *Proc. Natl. Acad. Sci. U. S. A.* 98, 75–80

23 Fitzgerald, K. (2000) In vitro display technologies – new tools for drug discovery. *Drug Discov. Today* 5, 253–257

24 Roberts, R. and Szostak, J. (1997) RNA-peptide fusions for the in vitro selection of peptides and proteins. *Proc. Natl. Acad. Sci. U. S. A.* 94, 12297–12302

25 Kurz, M. et al. (2000) Psoralen photo-crosslinked mRNA-puromycin conjugates: a novel template for the rapid and facile preparation of mRNA-protein fusions. *Nucleic Acids Res.* 28, E83

26 Kurz, M. et al. (2001) cDNA - protein fusions: covalent protein - gene conjugates for the in vitro selection of peptides and proteins. *Chem. Biochem* 2, 666–672

27 Schnos, M. and Inman, R.B. (1971) Starting point and direction of replication in P2 DNA. *J. Mol. Biol.* 55, 31–38

28 Geisselsoder, J. (1976) Strand-specific discontinuity in replicating P2 DNA. *J. Mol. Biol.* 100, 13–22

29 Chattoraj, D.K. (1978) Strand-specific break near the origin of bacteriophage P2 DNA replication. *Proc. Natl. Acad. Sci. U. S. A.* 75, 1685–1689

30 Weng, S. et al. (2002) Generating addressable protein microarrays with PROfusion covalent mRNA-protein fusion technology. *Proteomics* 2, 48–57

The *BioMedNet Magazine*

The online-only *BioMedNet Magazine* contains a range of topical articles currently available in *Current Opinion* and *Trends* journals, and offers the latest information and observations of direct and vital interest to researchers.

You can elect to receive the *BioMedNet Magazine* delivered directly to your email address, for a regular and convenient survey of at what's happening outside your lab, your department, your specialty.

Issue by issue, the *BioMedNet Magazine* provides an array of some of the finest material available on BioMedNet, dealing with matters of daily importance: careers, funding policies, current controversy and changing regulations in the practice of research.

Don't miss out!
Join the challenge at the start: register now at **http://news.bmn.com/magazine** to receive our regular editions.
Written with you in mind – the *BioMedNet Magazine*.

Samsung Bioepis Exhibit No. 1002
Page 968

LAWYERS TRAVEL

Thank you for choosing Lawyers Travel.

We value your input and welcome you to provide your feedback here.

Travel Summary **Agency Locator: ZBHEND**

Traveler
GASTONGUAY/ADAM
Reference number by traveler: 49571-09000

Date	From/To	Flight/Vendor	Depart/Arrive	Class/Type	Status
11/09/2016	BOS-DCA	B6 255	08:00 AM/09:44 AM	Economy	Confirmed
11/09/2016	DCA	Hyatt Regency Crystal City	11/09-11/10	See description below	Confirmed
11/10/2016	DCA-BOS	B6 790	03:35 PM/05:04 PM	Economy	Confirmed

Travel Assistance Contact Information

For travel assistance 24 hours a day, please call your dedicated number at 866-763-1399.
After business hours, weekends and holidays, you will have the option to be transferred to our after hours travel team.
To reach our after hours team directly in case of emergency, you may call 844-224-9301. Your access code is FE0C.

Travel Documentation Requirements

- A valid, government-issued photo ID is required for all domestic air travel.

AIR - Wednesday, November 9 2016

Jetblue Flight B6255 Economy Class

Depart:	Logan International, TERMINAL C Boston, Massachusetts, United States 08:00 AM Wednesday, November 9 2016
Arrive:	Ronald Reagan National, TERMINAL B Washington, District of Columbia, United States 09:44 AM Wednesday, November 9 2016
Duration:	1 hour(s) and 44 minute(s) Non-stop
Status:	Confirmed - Jetblue Booking Reference: DFTDHM
Equipment:	EMBRAER 190
Seat:	11B Confirmed
	For additional information and check in, 24 hours in advance, click on the airline link below. **http://www.jetblue.com/**

HOTEL - Wednesday, November 9 2016

Hyatt Regency Crystal City

Address:	2799 Jefferson Davis Hwy Arlington, VA 22202 United States
Tel:	+1 (703) 418-1234
Fax:	+1 (703) 418-1289
Check In/Check Out:	Wednesday, November 9 2016 - Thursday, November 10 2016
Status:	Confirmed
Confirmation:	HY0028959240
Number of Persons:	1
Number of Nights:	1
Number of Rooms:	1
Rate per night:	USD 199.00 plus tax and/or additional fees
Guaranteed:	Yes
Additional Information:	RQST NON SMK KING BED
Description:	BCD TRAVEL 1 KING BED CITY AIRPORT VIEW
Remarks:	CANCEL 24 HOURS PRIOR TO ARRIVAL TO AVOID PENALTY

AIR - Thursday, November 10 2016

Jetblue Flight B6790 Economy Class

Depart:	Ronald Reagan National, TERMINAL B Washington, District of Columbia, United States 03:35 PM Thursday, November 10 2016
Arrive:	Logan International, TERMINAL C Boston, Massachusetts, United States 05:04 PM Thursday, November 10 2016
Duration:	1 hour(s) and 29 minute(s) Non-stop
Status:	Confirmed - Jetblue Booking Reference: DFTDHM
Equipment:	EMBRAER 190
Seat:	20C Confirmed
	For additional information and check in, 24 hours in advance, click on the airline link below. **http://www.jetblue.com/**

Invoice / Ticket Receipt

Total Invoiced Amount: $294.20 USD

Transaction Date: October 20, 2016

Ticket Information

Ticket Number: 2797871532960
Amount: $256.20 USD
Form of Payment: VI**********5598

This ticket information applies to the following travel segment(s):
Jetblue Flight 255 from Boston to Washington on November 09
Jetblue Flight 790 from Washington to Boston on November 10

Transaction Date: October 20, 2016

Service Fee Information

Service Fee Number: 8900696459360
Service Fee Amount: $38.00 USD
Form of Payment: VI**********5598
Service Fee Description: Air Ticket

Information Specific to this Trip

- You have purchased a non-refundable fare on **Jetblue**. Any changes are subject to change fee plus any fare increase.

Other Information and Remarks

- Please sign up for trip alerts at www.lawyerstravel.com/alerts.
- Please reconfirm all flight times prior to your departure.
- Please notify us of any trip cancellation so your tickets may be refunded or logged for future use.
- Some hotels may impose a penalty for early checkout.

Important Health Advisory

CDC has issued travel notices for people traveling to regions where Zika virus transmission is ongoing. For more information please visit http://www.cdc.gov/zika.

TripCase

Reservation management tool and mobile app

If you prefer to view this reservation on TripCase.com and have a TripCase account, you may do so by forwarding this email to trips@tripcase.com or, if your TripCase email address matches the one we have for this reservation, this trip will automatically appear in the "My Trips" section of your TripCase account.

This invoice was generated Thursday, October 20, 2016 7:29 PM UTC. Agency Locator: ZBHEND

Other News To Note

Publication info: BIOWORLD Today (Aug 9, 2002): NA.
ProQuest document link

Abstract (English): Accelrys Inc., of San Diego, reported the availability of five Accelrys software products on IBM eServer pSeries systems running AIX. These are the first product releases stemming from the global strategic alliance between IBM and Accelrys. The applications are geared toward drug discovery and bioinformatics.
Alexion Pharmaceuticals Inc., of Cheshire, Conn., completed enrollment in its first Phase II trial in patients with a severe form of kidney disease, known as membranous nephritis, who are being treated with its anti-inflammatory C5 inhibitor antibody, 5G1.1, now called eculizumab. Separately, Alexion received orphan drug designation from the FDA for the use of eculizumab for this disease. The Phase II multicenter, double-blinded, randomized, placebo-controlled study examines the safety and efficacy of repeated doses of eculizumab over four months.

Full text: Accelrys Inc., of San Diego, reported the availability of five Accelrys software products on IBM eServer pSeries systems running AIX. These are the first product releases stemming from the global strategic alliance between IBM and Accelrys. The applications are geared toward drug discovery and bioinformatics.
Alexion Pharmaceuticals Inc., of Cheshire, Conn., completed enrollment in its first Phase II trial in patients with a severe form of kidney disease, known as membranous nephritis, who are being treated with its anti-inflammatory C5 inhibitor antibody, 5G1.1, now called eculizumab. Separately, Alexion received orphan drug designation from the FDA for the use of eculizumab for this disease. The Phase II multicenter, double-blinded, randomized, placebo-controlled study examines the safety and efficacy of repeated doses of eculizumab over four months. Alexion also said it began dosing in a follow-on, open-label extension study of the antibody in these patients, designed to examine long-term safety and tolerability.
Alkermes Inc., of Cambridge, Mass., signed an agreement with Indianapolis-based Eli Lilly &Co. that provides for an investment by Lilly in AlkermesE commercial-scale production facility for inhaled pharmaceutical products based on AlkermesE AIR pulmonary drug delivery technology. The facility will have the capability to produce multiple products and is under construction in Chelsea, Mass. Alkermes and Lilly are collaborating on the development of inhaled formulations of insulin for diabetes. Lilly will make an investment in the Chelsea facility to fund pulmonary insulin production and packaging capabilities. If inhaled formulations of insulin are developed successfully and are commercially launched, Lilly will purchase product from the facility. (See BioWorld Today, April 3, 2001.)
Amarin Corp. plc, of London, said its subsidiary, Amarin Development AB, of Malmo, Sweden, will establish a strategic technology partnership with NanoCarrier Co. Ltd., of Chiba, Japan. The partnershipEs goal is to develop controlled-release technologies for use in pharmaceutical products, and the companies will share the costs of development together with any resulting commercial benefits. Initially, the collaboration will focus on the feasibility of developing a technology for the oral controlled release of nanoparticles, using AmarinEs Diffusion Controlled Vesicle controlled-release technology and NanoCarrierEs micellar nanoparticle controlled-release technology.
Amylin Pharmaceuticals Inc., of San Diego, reported the exercise by its underwriters of an overallotment option to purchase 1.57 million shares of common stock associated with its follow-on stock offering that priced on Feb. 11. Including the overallotment shares, the offering totaled 12 million shares at $8 per share, resulting in expected net proceeds to the company of about $91 million. The joint lead managers for the offering were Goldman Sachs and Lehman Brothers. (See BioWorld Today, Jan. 31, 2002, and Feb. 13, 2002.)
Applied Genetic Technologies Corp., of Alachua, Fla., completed a license agreement with Johns Hopkins

University in Baltimore for use of adeno-associated virus gene therapy vectors in muscle tissue. The agreement gives AGTC the right to develop the AAV technology for injection into human muscle tissue for potential therapy of alpha-l antitrypsin deficiency and PompeEs disease.

AtheroGenics Inc., of Atlanta, completed a Phase I trial demonstrating the safety of AGIX-4207, its lead oral compound for rheumatoid arthritis. The results in 48 healthy volunteers indicated that it was well tolerated over the single and multiple dose ranges studied. AtheroGenics plans to initiate a Phase II efficacy study in patients with rheumatoid arthritis in the second quarter.

BioMicro Systems Inc., of Salt Lake City, entered agreements with the National Cancer Institute, the Huntsman Cancer Institute and the Buck Institute for Age Research to evaluate its Microarray User Interface (MAUI) hybridization product. The alpha sites will test BioMicroEs MAUI microfluid analysis product used to expedite the drug discovery process by obtaining results from microarray hybridization.

Atugen AG, of Berlin, signed a technology evaluation agreement with Kinetek Pharmaceuticals Inc., of Vancouver, British Columbia, which focuses on the discovery and development of selective signal transduction inhibitors in cancer and inflammatory diseases. Kinetek will evaluate the application of AtugenEs target-validation technology in its research and development efforts in oncology. Atugen will optimize its GeneBloc and cellular delivery technologies in specific cell lines to knock down the expression of a number of signal transduction kinase targets identified by Kinetek. Subsequently, Kinetek will use the optimized GeneBloc molecules to validate the gene function of these targets using its proteomics technologies. Financial details were not disclosed.

Bayer AG, of Leverkusen, Germany, and Aventis Pharma SA, of Antony, France, signed a nonbinding letter of intent to establish a joint venture for biological products. The companies plan to combine BayerEs Biological Products Business Group with AventisE subsidiary, Aventis Behring. Bayer will own a majority interest and have operational control. Included are the marketing rights to factor VIII products that will continue to be manufactured by Bayer using genetic engineering techniques. Bayer will have the option to acquire the remaining interest at a later date. BayerEs biological group, headquartered in Research Triangle Park, N.C., has products that are derived from blood plasma. Aventis Behring focuses its research and development efforts on proteins from human blood plasma.

BioSeek Inc., of Burlingame, Calif., raised $8.4 million in its first round of venture financing. The Series A round investors included Bay City Capital, Fremont Ventures, SImile Investors LLC and Vanguard Ventures. BioSeekEs president and CEO said the money will allow it to aggressively develop and exploit its platforms for functional genomics and drug discovery.

Chiron Corp., of Emeryville, Calif., completed a cash tender offer by Manon Acquisition Corp., a wholly owned subsidiary of Chiron Corp., for all the outstanding common stock of Matrix Pharmaceutical Inc., of Fremont, Calif. The tender offer expired as scheduled on Tuesday. Based on preliminary information, a total of 22.7 million shares representing about 86 percent of the outstanding common stock of Matrix were validly tendered, Chiron said. Chiron, Manon and Matrix entered into a merger agreement pursuant to which Manon began on Jan. 14 an all-cash tender offer for all of MatrixEs outstanding common stock at $2.21 per share. (See BioWorld Today, Jan. 8, 2002.)

ChromaVision Medical Systems Inc., of San Juan Capistrano, Calif., entered an agreement with Abbott Laboratories, of Abbott Park, Ill., to support the clinical development of AbbottEs cancer compounds targeting tumor angiogenesis. ChromaVisionEs imaging solution technology, Automated Cellular Imaging System, rare-event detection capability and ChromaVision-labeled antibodies and reagents will be employed in the research and assessment of the efficacy of AbbottEs developmental anti-angiogenic compounds.

Ciphergen Biosystems Inc., of Fremont, Calif., said it sold multiple ProteinChips systems to hospitals, universities and government agencies in China, which intend to focus their initial use of the systems on protein expression profiling in a variety of diseases to develop predictive diagnostic tests. Ciphergen also established a

formal relationship with the Universities Confederated Institute for Proteomics, established by the Chinese government in May 2001. The company also agreed to provide priority technical assistance to UCIP members, which include many of the medical colleges and universities in China.

CombiMatrix Corp., of Mukilteo, Wash., a majority-owned subsidiary of Acacia Research Corp., was awarded a Phase I National Institutes of Health grant for the development of its protein biochip technology. The title of the grant is oSelf-Assembling Protein Microchips.o The grant is in addition to a three-year Phase I and a Phase II Small Business Innovation Research grant from the U.S. Department of Defense for the development of multiplexed chip-based assays for chemical and biological warfare agent detection.

EraGen Biosciences Inc., of Madison, Wis., named Irene Hrusovsky president and CEO and appointed her to the board. She is a former executive of GlaxoSmithKline plc, of London, where she served as vice president, general medicine/medical marketing strategy, clinical operations. Prior to her position at GSK, Hrusovsky spent more than 20 years at Abbott Laboratories, of Abbott Park, Ill. EraGen licenses bioinformatics, drug screening platforms, nucleic acid and genotyping technologies for clinical diagnostic and life science research applications.

Illumina Inc., of San Diego, signed an agreement with Amersham Biosciences, the life sciences business of Amersham plc, of London, granting Illumina a nonexclusive worldwide license under five fundamental patents involving engineering designs and methods for confocal scanning for use with its BeadArray product line. Additional terms of the agreement were not disclosed.

ImmunoGen Inc., of Cambridge, Mass., and Millennium Pharmaceuticals Inc., of Cambridge, Mass., signed an exclusive product license for use of ImmunoGenEs maytansinoid tumor-activated prodrug (TAP) technology with MillenniumEs MLN591 antibody. This triggers MillenniumEs payment of a product-licensing fee to ImmunoGen. In March 2001, the companies announced the formation of a collaboration that provides Millennium with access to ImmunoGenEs TAP technology and enables Millennium to obtain exclusive product licenses for a restricted number of specific antigen targets. MLN591 is directed toward the extracellular domain of the prostate-specific membrane antigen. ImmunoGenEs TAP technology couples the companyEs anticancer agents with tumor-targeting antibodies to achieve antibody-directed killing of cancer cells. (See BioWorld Today, March 7, 2001.)

Molecular Diagnostics Inc., of Chicago, and Esoterix Inc., of Austin, Texas, said Esoterix will use Molecular DiagnosticsE In-Cell HPV probe for an upcoming clinical trial of new approaches to identify patients with cervical cancer. The In-Cell HPV probes are designed to detect messenger RNA of the E6 and E7 oncogenes, and provide an alternative to HPV DNA testing to screen women with HPV infections.

ProteoTech Inc., of Kirkland, Wash., received funding totaling $2.78 million from the National Institute of Aging for two research programs. The programs are designed to lead to therapeutics for the prevention and treatment of brain amyloid protein deposits present in patients with AlzheimerEs disease. It also is designed to create a closer understanding of the mechanisms involved in AlzheimerEs amyloid brain plaque formation and persistence.

Samaritan Pharmaceuticals Inc., of Las Vegas, said that Georgetown University in Washington notified Samaritan of the discovery of a potential therapeutic for AlzheimerEs disease, and Samaritan licensed the product. This is the first discovery to result from the collaboration. Samaritan initiated a seven-year contract to fund Samaritan Research Laboratories at Georgetown in July 2001.

Synthetic Blood International Inc., of Costa Mesa, Calif., said that company officials are planning to meet with the FDA before the end of April to review plans to file an investigational new drug application for Phase I clinical trials with Oxycyte. The company said it expects the IND to be filed by the end of the year. Oxycyte, a blood substitute, is one of two perfluorocarbon-based products being developed by the company.

Synthon Chiragenics Corp., of Monmouth Junction, N.J., introduced DiscoveryCollections, a program that makes SynthonEs chemistry platforms available to companies for independent research. Four collections

Diamond, Platinum, Gold and Silver will consist of palettes of compounds representing different aspects of a technology platform for drug discovery.

Target Discovery Inc., of Palo Alto, Calif., raised more than $4.6 million in Series A financing for its proteomics platform technologies for comparison and identification of differences in the protein makeup of healthy and diseased tissues. Clayton Struve, its largest investor, was joined by other private angel investors from the Chicago area.

U.S. Genomics Inc., of Woburn, Mass., established a research collaboration with Pui-Yan Kwok, a researcher focused on genetics and technology at Washington University School of Medicine in St. Louis. KwokEs laboratory will employ U.S. GenomicsE direct, linear DNA analysis technology for multiple applications. Kwok also will work to extend the development of the technology platform toward additional applications in the field of genetics. Financial terms were not disclosed.

Xencor Inc., of Monrovia, Calif., signed licensing agreements with the California Institute of Technology and Pennsylvania State University to further expand its protein design automation platform. The agreements expand the patent estate of the PDA technology and increase XencorEs computational throughput and accuracy in protein optimization. Xencor said the PDA technology is the first and only protein optimization method to combine advanced computational methods, high-performance computing and experimental screening.

Subject: Alliances and partnerships;Biological products;Computer industry;Computer industry -- Alliances and partnerships;Microcomputer industry;Pharmaceutical industry -- Alliances and partnerships

Location: United States

Company / organization: International Business Machines Corp.; Alexion Pharmaceuticals Inc.; International Business Machines Corp. (Alliances and partnerships); Alexion Pharmaceuticals Inc. (Alliances and partnerships)

Identifier (keyword): Trade, IBM, ALXN

Event classification: 389: Alliances, partnerships

NAICS classification: 325414: Biological Product (except Diagnostic) Manufacturing

Product classification: 2831000: Biological Products

SIC classification: 2834: Pharmaceutical preparations;3571: Electronic computers;2833: Medicinals and botanicals

Publication title: BIOWORLD Today

Pagination: NA

Publication date: Aug 9, 2002

Publisher: AHC Media LLC

Journal subject: Biotechnology industry, Business

ISSN: 1541-0595

Journal code: 0EUH

Source type: Magazine/Journal

Language of publication: English

Document type: Magazine/Journal

Source attribution: Gale Group Newsletter Database, © Publisher specific

Accession number: 211122339

Document URL: http://dialog.proquest.com/professional/docview/1074897658?accountid=157282

Copyright: Copyright 2002 Gale, Cengage Learning. All rights reserved.COPYRIGHT 2002 A Thomson Healthcare Company

Date created: 2012-01-27

Updates: 2012-09-26

First available: 2012-09-26

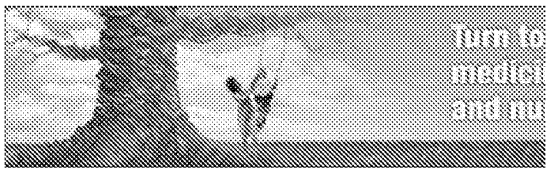
Database: Gale Group Newsletter Database™ (1988 - current)

Contact ProQuest

Copyright © 2016 ProQuest LLC. All rights reserved. - **Terms and Conditions**

Wiley Online Library

Log in / Register



Go to old article view

Arthritis & Rheumatology *Explore this journal >*

View Issue TOC
Volume 52, Issue 7
July 2005
Pages 2120–2124

Research Article

Requirement of activation of complement C3 and C5 for antiphospholipid antibody–mediated thrombophilia

Silvia S. Pierangeli , Guillermina Girardi, Mariano Vega-Ostertag, Xiaowei Liu, Ricardo G. Espinola, Jane Salmon

First published:
28 June 2005 Full publication history

DOI:
10.1002/art.21157 View/save citation

Cited by:
0 articles Citation tools

Funding information

Abstract

Objective

Antiphospholipid antibodies (aPL) have been shown to induce thrombosis, activate endothelial cells, and induce fetal loss. The pathogenesis of aPL-induced thrombosis, although not completely understood, may involve platelet and endothelial cell activation as well as procoagulant effects of aPL directly on clotting pathway components. Recent studies have shown that uncontrolled complement activation leads to fetal death in aPL-treated mice. In this study, we tested the

Samsung Bioepis Exhibit No. 1002
Page 977

hypothesis that aPL are responsible for activation of complement, thus generating split products that induce thrombosis.

Methods

To study thrombus dynamics and adhesion of leukocytes we used in vivo murine models of thrombosis and microcirculation, in which injections of aPL were used.

Results

Mice deficient in complement components C3 and C5 were resistant to the enhanced thrombosis and endothelial cell activation that was induced by aPL. Furthermore, inhibition of C5 activation using anti-C5 monoclonal antibodies prevented thrombophilia induced by aPL.

Conclusion

These data show that complement activation mediates 2 important effectors of aPL, induction of thrombosis and activation of endothelial cells.

Antiphospholipid syndrome (APS) is characterized by increased risk of vascular thrombosis, involving the venous, arterial, and placental circulatory systems. The pathogenic mechanisms for antiphospholipid antibody (aPL)–induced thrombosis are incompletely understood. Passive transfer of IgG from aPL-positive sera (IgG-APS) has been found to induce fetal loss, thrombosis, and endothelial cell activation in mice, suggesting a direct pathogenic role of aPL (1–3). Complement activation is a necessary intermediary event in the pathogenesis of fetal loss associated with aPL in this model (4, 5).

It is well established that activated complement fragments themselves have the capacity to bind and activate inflammatory and endothelial cells, as well as induce a prothrombotic phenotype either directly through C5b–9 (membrane attack complex [MAC]) or through C5a receptor (C5aR)–mediated effects (6, 7). Endothelial cells can release tissue factor in response to C5a activation (8). Inflammatory cells, when triggered by complement proteolytic products C5a and C3a, respond with the production of selected procoagulant activities, thereby initiating the coagulation pathways. MAC has also been associated with thrombosis. Studies performed in rats showed that CD59, an inhibitor of C5b–9 assembly and insertion, serves a protective role in a rat model of thrombotic microangiopathy, demonstrating that C5b–9 plays a critical role in the pathogenesis of thrombosis (9).

In previous studies, we demonstrated that the complement C3 convertase inhibitor, Crry, inhibited IgG -APS–induced thrombosis, suggesting that complement activation is required in IgG-APS–induced thrombophilia (4). Moreover, Girardi and coworkers proposed that heparin, the current standard treatment in patients with APS, prevents obstetric complications by blocking activation of complement, as opposed to preventing placental thrombosis (10). We therefore tested the hypothesis that complement activation mediates endothelial cell activation and the thrombogenic effects of IgG-APS.

Samsung Bioepis Exhibit No. 1002
Page 978

MATERIALS AND METHODS

Mice.

C3-deficient mice were obtained from V. M. Holers (University of Colorado Health Sciences, Denver) (11). The mice were generated by intercrossing $C3^{+/-}$ mice at F1 during a backcross to C57BL/6 and then propagating $C3^{-/-}$ progeny. $C5^{-/-}$ mice (B10.D2-$H2^{d}$H2-T18^{c} Hc^{0}/o2Sn) and the $C5^{+/+}$ background strain of mice (B10.D2-$H2^{d}$H2-T18^{c} Hc^{0}/oSnJ) were obtained from The Jackson Laboratories (Bar Harbor, ME). CD1 male mice (weight ~30 grams) were obtained from Charles River Laboratories (Wilmington, DE). All animals were housed in the animal care (American Association of Laboratory Animal Care–approved) facilities of the Morehouse School of Medicine. Animals were handled by trained personnel according to Institutional Animal Care and Use Committee guidelines.

Preparation of IgG-APS.

Antiphospholipid antibodies from APS patients (IgG-APS) were affinity-purified using cardiolipin liposomes and protein G–Sepharose chromatography as previously described (2, 3). Human IgG from a non-autoimmune healthy individual (IgG-NHS) was purified by an identical method. The sterile-filtered IgG fractions were determined to be free of endotoxin contamination by the limulus amoebocyte lysate assay (E-Toxate; Sigma, St. Louis, MO). Protein concentration was determined using the method of Lowry (12). Levels of human anticardiolipin (aCL) and anti–β_2-glycoprotein I antibodies were measured by standard enzyme-linked immunosorbent assay, performed as previously described (2, 3).

Analysis of thrombus dynamics and leukocyte adhesion in complement-deficient mice.

To investigate the role of C3 and C5 in thrombophilia induced by aPL, $C3^{+/+}$ and $C3^{-/-}$ mice (n = 7–10 animals) and $C5^{+/+}$ and $C5^{-/-}$ mice (n = 7–10 animals) were injected intraperitoneally with 500 μg of IgG-APS or with 500 μg of IgG-NHS twice, at time 0 and at 48 hours later. Surgical procedures to study thrombus dynamics and adhesion of leukocytes to the endothelium of postcapillary venules (number of adhering white blood cells [WBCs]) in the exposed cremaster muscle were performed 72 hours after the first IgG-APS or IgG-NHS injection, as described previously (2, 3). The mouse model of thrombosis formation used in this study has previously been described in detail (2, 3). Briefly, mice were anesthetized and the right femoral vein was exposed, resulting in a 0.5-cm segment of vein free for manipulation and observation. The vein was pinched with a pressure of 1,500 gm/mm^2 to introduce a standardized injury that induced a clot. Clot formation and dissolution in the transilluminated vein were visualized with a microscope equipped with a closed-circuit video system (including a color monitor and a recorder). Thrombus size (expressed in μm^2) was measured 1 minute after the pinch injury by freezing the digitized image and tracing the outer margin of the thrombus. Three-to-five thrombi were successfully induced in each animal, and mean values were computed.

Adhesion of leukocytes to endothelium in the cremaster muscle is an indication of endothelial cell activation (3). The number of leukocytes (WBCs) adhering within 5 different venules in the cremaster

Samsung Bioepis Exhibit No. 1002

muscle was determined, and adhesion was defined as the presence of WBCs that remained stationary for at least 30 seconds (2, 3).

Analysis of thrombus dynamics using the anti-C5 monoclonal antibody (mAb).

Anti-C5 mAb have been shown to prevent C5 activation in vivo and in vitro and to protect mice from aPL-induced pregnancy loss (5, 13). To further investigate the role of C5 in thrombophilia induced by aPL, male CD1 mice (weight 25–30 grams; Charles River Laboratories) were injected intraperitoneally with 500 μg of IgG-APS or IgG-NHS (n = 10 mice per group) at time 0 and at 48 hours later. Half of the mice in each group received 1 mg of anti-C5 mAb (BB5.1) and half received 1 mg of murine IgG control at 30 minutes before each injection with IgG-APS or IgG-NHS. Surgical procedures to study thrombus dynamics were performed 72 hours after the first IgG-APS (or IgG-NHS) injection, as previously described (2, 3).

Statistical analysis.

An independent *t*-test was used to compare the antibody levels in different groups of mice. Student's unpaired *t*-test was used to compare the mean thrombus size and number of adhering WBCs between treated and control groups. *P* values of less than 0.05 were considered significant.

RESULTS

Protection of C3- and C5-deficient mice from IgG-APS–induced thrombophilia in vivo.

Consistent with our previous findings, IgG-APS significantly enhanced thrombus size (3.3-fold increase) in $C3^{+/+}$ mice when compared with the effects of treatment with IgG-NHS in $C3^{+/+}$ mice (2, 4). In $C3^{-/-}$ mice treated with IgG-APS, there was a significant reduction ($P = 0.0002$) in the size of injury-induced thrombi (67% decrease) when compared with the effects of IgG-APS in $C3^{+/+}$ mice (Table 1). This reduction in thrombus size in $C3^{-/-}$ mice treated with IgG-APS was similar to that in $C3^{-/-}$ or $C3^{+/+}$ mice treated with IgG-NHS (Table 1).

Table 1. Effects of IgG-APS on thrombus size and leukocyte adhesion to endothelial cells in $C3^{-/-}$ mice*

Mouse type, treatment	No. of animals	Thrombus size, μm^2	WBC adhesion	aCL titer, GPL units†

* Values are the mean ± SD. IgG-APS = IgG from antiphospholipid-positive sera; WBC = white blood cell (expressed as no. of leukocytes adhering within 5 different venules); aCL = anticardiolipin; GPL = IgG phospholipid; WT = wild-type; IgG-NHS = IgG from non-autoimmune healthy sera; C3−/− = deficient in C3.

† Negative titer considered <10 GPL units.

Samsung Bioepis Exhibit No. 1002
Page 980

Mouse type, treatment	No. of animals	Thrombus size, μm²	WBC adhesion	aCL titer, GPL units†
WT				
IgG-NHS	9	802 ± 390	14.0 ± 5.0	<10
IgG-APS	7	2,646 ± 980	35.0 ± 12.0	78.0 ± 8.6
C3$^{-/-}$				
IgG-NHS	7	1,083 ± 443	4.7 ± 0.9	<10
IgG-APS	7	873 ± 425	4.7 ± 2.0	86.3 ± 10.4

* Values are the mean ± SD. IgG-APS = IgG from antiphospholipid-positive sera; WBC = white blood cell (expressed as no. of leukocytes adhering within 5 different venules); aCL = anticardiolipin; GPL = IgG phospholipid; WT = wild-type; IgG-NHS = IgG from non-autoimmune healthy sera; C3−/− = deficient in C3.

† Negative titer considered <10 GPL units.

In C5$^{+/+}$ mice, IgG-APS induced a significant, 2.3-fold increase in thrombus size ($P = 0.003$) when compared with the effects of IgG-NHS in C5$^{+/+}$ mice. The increase in thrombus size induced by IgG-APS was reduced by 54% ($P = 0.011$) in C5$^{-/-}$ mice (Table 2). The thrombus size in C5$^{-/-}$ mice treated with IgG-APS was not different from that in C5$^{-/-}$ or C5$^{+/+}$ mice treated with IgG-NHS (Table 2).

Table 2. Effects of IgG-APS on thrombus size and leukocyte adhesion to endothelial cells in C5$^{-/-}$ mice*

Mouse type, treatment	No. of animals	Thrombus size, μm²	WBC adhesion	aCL titer, GPL units†
WT				
IgG-NHS	7	447 ± 142	1.4 ± 0.4	<10
IgG-APS	10	1,066 ± 141	6.5 ± 2.5	115.7 ± 74.2
C5$^{-/-}$				
IgG-NHS	7	303 ± 248	1.4 ± 0.4	<10
IgG-APS	7	493 ± 149	1.7 ± 0.5	102.3 ± 12.2

* Values are the mean ± SD. See Table 1 for definitions.

† Negative titer considered <10 GPL units.

Samsung Bioepis Exhibit No. 1002
Page 981

Protection of C3- and C5-deficient mice from IgG-APS–induced endothelial cell activation in vivo.

To determine whether complement is required for aPL induction of in vivo endothelial cell activation, we examined WBC adhesion to the endothelium of cremaster muscle. IgG-APS induced an increase in WBC adhesion in both $C3^{+/+}$ and $C5^{+/+}$ mice (2.5-fold [$P = 0.01$] and 4.6-fold [$P = 0.0025$], respectively) when compared with the effects of treatment with IgG-NHS (Tables 1 and 2). In contrast, adhesion of WBCs to cremaster endothelium was significantly reduced after treatment with IgG-APS in C3- and C5-deficient mice (reduction of 87% [$P = 0.002$] and 74% [$P = 0.003$], respectively) (Tables 1 and 2).

The titer of aCL antibodies in $C3^{+/+}$ mice injected with IgG-APS was not different from the aCL titers in $C3^{-/-}$ mice treated with IgG-APS (mean ± SD 78 ± 8.6 IgG phospholipid [GPL] units versus 86.3 ± 10.4 GPL units). Similarly, the levels of aCL antibodies in $C5^{+/+}$ mice treated with IgG-APS were not different from the aCL levels in $C5^{-/-}$ mice treated with IgG-APS (115.7 ± 74.2 GPL units versus 102.3 ± 12.2 GPL units). These findings exclude the possibility that the protective effects of IgG-APS in reducing proinflammatory and thrombogenic events in $C3^{-/-}$ and $C5^{-/-}$ mice were related to lower aCL titers in these mice.

Prevention of IgG-APS–induced thrombosis by anti-C5 mAb.

To confirm that C5 activation is required for the induction of thrombosis by aPL, mice treated with IgG-APS were injected with anti-C5 mAb to inhibit C5 cleavage. In control mice, IgG-APS caused an increase in thrombus size (mean ± SD 3,577 ± 1,129 μm^2 with IgG-APS versus 712.0 ± 272 μm^2 with IgG-NHS; $P = 0.001$) (Figure 1), whereas in mice treated with anti-C5 mAb and IgG-APS, the thrombus size was significantly smaller (838 ± 222 μm^2; $P = 0.005$) when compared with mice treated with control IgG and IgG-APS (Figure 1). The mouse IgG used as the control for the anti-C5 mAb did not affect thrombus size in animals treated with IgG-APS or IgG-NHS. The aCL titer in IgG-APS–treated mice was 152.2 ± 14.8 GPL units and was not different from the aCL titer in mice treated with IgG-APS and anti-C5 mAb (135.3 ± 20.3 GPL units), thus excluding the possibility that the protective effects of anti-C5 mAb were due to a diminution in aCL titers because of interferences between IgG-APS and anti-C5 mAb.

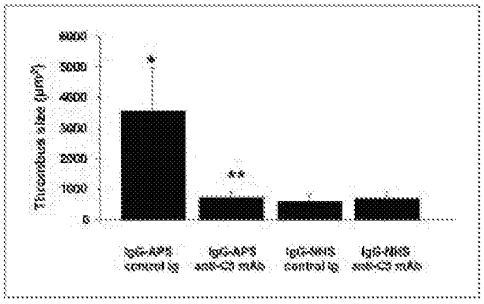


Figure 1.

Open in figure viewer

Samsung Bioepis Exhibit No. 1002

Effects of anti-C5 monoclonal antibody (mAb) on thrombosis induced by IgG from patients with antiphospholipid syndrome (IgG-APS). CD1 mice (5 per group) were injected with 1 mg of anti-C5 mAb or 1 mg of murine IgG control. After 30 minutes, the mice were injected with IgG-APS or with IgG from a non-autoimmune healthy individual (IgG-NHS) twice (at time 0 and 48 hours later). The size of induced thrombi (in μm^2) was measured as described in Materials and Methods. Bars show the mean and SEM. $* = P = 0.001$, IgG-APS control Ig versus IgG-NHS control Ig; $** = P = 0.005$, IgG-APS anti-C5 mAb versus IgG-APS control Ig.

DISCUSSION

Using a model of surgically induced thrombus formation, we demonstrated that complement activation plays an important role in thrombosis induced by aPL in mice. Specifically, we identified C3 and C5 as the critical intermediaries linking pathogenic aPL to WBC adhesion and development of thrombosis. Our conclusions are based on the prevention of thrombophilia observed in $C3^{-/-}$ and $C5^{-/-}$ mice and the protective effects of anti-C5 mAb. In previous studies from our group, the findings suggested that C3 activation is required for aPL-induced thrombosis. We demonstrated that Crry-Ig, an inhibitor of C3 convertase, blocks thrombosis initiated by aPL (4). Subsequently, Girardi et al showed that complement activation, specifically C5a–C5aR interaction, is required for aPL-induced pregnancy loss and suggested that C5a promotes neutrophil infiltration of decidual tissue (5). Evidence that neutrophils activated by C5a release procoagulant substances and that monocytes activated by C5a release tissue factor suggests that infiltrating leukocytes stimulated by complement split products can initiate placental infarction and ultimately cause fetal death (8).

In the current study, we have extended our findings by demonstrating that complement is required for aPL-mediated thrombosis and for increased leukocyte adhesion to endothelium. In the absence of C3 or C5, we observed neither enhanced leukocyte adherence nor increased thrombosis associated with aPL treatment. Furthermore, we found that anti-C5 mAb prevented aPL-mediated thrombosis, emphasizing the role of C5 (either C5a, C5aR, or C5b–9) in induction of thrombophilia. C5a binding to endothelial cells results in increased expression of P-selectin and markedly increases neutrophil adhesion (14), and binding of C5b to target surfaces initiates assembly of the MAC that triggers proinflammatory signaling pathways and induces a prothrombotic phenotype in vascular tissue (8). Observations that blockade of C5aR prevents thrombus formation and leukocyte accumulation in a rat model of antibody-mediated thrombotic glomerulonephritis underscore the linkage between complement activation and thrombophilia (15).

Thrombosis in APS is sporadic and may occur in any vein or artery of the body. In this study, we used a mouse model of thrombosis induced by a standardized pinch injury in the femoral vein to define the mediators of thrombophilia associated with aPL (2, 3). Recently, other investigators have demonstrated enhancement of thrombosis by aPL in an experimental model of photochemically induced vascular injury in hamsters (16). Patients with APS often have aPL for prolonged periods of time without clinical manifestations, and thrombosis occurs after a triggering event such as an infection, immobilization, or surgery. Therefore, our experimental model of injury-induced thrombosis,

Samsung Bioepis Exhibit No. 1002

although artificial, simulates a "second hit" that triggers thrombotic episodes in susceptible patients and mimics sporadic clotting as observed clinically in APS.

We recognize that there are differences between the human and the murine complement systems. However, the anti-C5 mAb (BB5.1) used in these studies has been shown to effectively block C5 activation in vitro and in vivo in mice and in humans (13, 17, 18). Independent of the initiator of the complement cascade, this mAb prevents C5 activation and thus prevents the generation of the potent proinflammatory factors, C5a and C5b–9. Anti-C5 mAb precipitates the 2 chains of C5 from normal mouse serum and inhibits C5-dependent hemolysis in a functional complement test. It has been shown to prevent aPL-induced pregnancy loss, in which thrombosis plays an important role (8).

Anti-C5 biologic therapy has been extensively investigated in several other animal models of complement-mediated diseases, including collagen-induced arthritis and lupus-like autoimmune disease in (NZB/NZW)F_1 mice (19, 20). Eculizumab (5G1.1), the humanized anti-C5 mAb, is considered a potential treatment for several chronic inflammatory diseases, including rheumatoid arthritis and nephritis, and phase II trials have been initiated for these indications. Furthermore, eculizumab has been shown to prevent C5 activation in humans and to have beneficial effects in patients with paroxysmal nocturnal hemoglobinuria; specifically, it reduces intravascular hemolysis, hemoglobinuria, and the need for transfusion in these patients, providing a proof-of-concept that blockade of complement activation is feasible and tolerable in patients with chronic disease (18).

We propose that pathogenic aPL, in addition to their direct effects on platelet and endothelial cell targets, induce complement activation, and thus generate complement split products that attract inflammatory cells and initiate thrombosis and tissue injury. Our finding that blockade of C5 is effective in preventing thrombosis in a mouse model of APS has important therapeutic implications. Blockade of complement activation may be a valuable target for interventions that prevent, arrest, or modify the thrombogenic effects of aPL.

REFERENCES

Related content

WILEY

Browse Publications
Browse by Subject
Resources
Help & Support
Cookies & Privacy
Terms & Conditions

Samsung Bioepis Exhibit No. 1002
Page 984

About Us
Wiley Job Network
Advertisers & Agents

Powered by Wiley Online Library Copyright © 1999 - 2016 John Wiley & Sons, Inc. All Rights Reserved

nature biotechnology

REVIEW

© 2007 Nature Publishing Group http://www.nature.com/naturebiotechnology

Complement-targeted therapeutics

Daniel Ricklin & John D Lambris

The complement system is a central component of innate immunity and bridges the innate to the adaptive immune response. However, it can also turn its destructive capabilities against host cells and is involved in numerous diseases and pathological conditions. Modulation of the complement system has been recognized as a promising strategy in drug discovery, and a large number of therapeutic modalities have been developed. However, successful marketing of complement-targeted drugs has proved to be more difficult than initially expected, and many strategies have been discontinued. The US Food and Drug Administration's approval of the first complement-specific drug, an antibody against complement component C5 (eculizumab; Soliris), in March 2007, was a long-awaited breakthrough in the field. Approval of eculizumab validates the complement system as therapeutic target and might facilitate clinical development of other promising drug candidates.

When the bactericidal properties of certain heat-labile blood components were initially described more than a century ago[1], no one could have imagined the considerable impact the complement system would have on both immunology and our understanding of many disease processes. Even the name "complement," originally coined by Paul Ehrlich[2], implied only a supplementary role of this system in the defense against microbial intruders. However, continued progress in research has led to a dramatic change in our knowledge about the complement system and moved it into the spotlight of basic and applied life sciences.

Today it is clear that complement is a key player of the innate immune system. However, this integral position in the maintenance and regulation of immune and inflammation reactions also makes it a trigger point for a variety of pathologic conditions. Erroneous activation or insufficient regulation of the complement cascade may turn its destructive actions against the host's cells. As a consequence, many inflammatory and autoimmune diseases are thought to be caused, or at least supported, by unleashed complement. Inhibition or modulation of complement activity has therefore been recognized as a promising therapeutic strategy for many years.

Although many creative attempts to design complement-specific drugs have been made in recent decades, their development from experimental concept to clinical product has faced many obstacles. In light of these complications, most of the major pharmaceutical companies seem to have abandoned their initial efforts to develop drugs that target complement. Despite its bitter taste, this decision has encouraged a series of small startup companies in the biotechnology field to take up the challenge. The first results of this commitment are now visible and may well lead to a revival of this difficult but promising area of drug discovery. With its 2007 approval of an antibody against complement component C5 (eculizumab; Alexion, Cheshire, CT, USA), the US Food and Drug Administration (FDA) has now authorized the first complement-specific drug[3]. Many other promising drug candidates and therapeutic strategies are currently in the pipeline of various companies. The aim of this review is to provide an overview of current therapeutic strategies, with a focus on drug candidates that are in clinical trials (**Table 1**) or late preclinical development (**Table 2**).

Department of Pathology and Laboratory Medicine, School of Medicine, University of Pennsylvania, 401 Stellar Chance, 422 Curie Blvd., Philadelphia, Pennsylvania 19104, USA. Correspondence should be addressed to J.D.L. (lambris@mail.med.upenn.edu).

Published online 7 November 2007; doi:10.1038/nbt1342

The complement system in innate and adaptive immunity

With an estimated age of 600–700 million years, the complement system is one of the most ancient defense strategies[4], yet it represents far more than merely a simple protection mechanism against microbial infections. As an essential connection between adaptive and innate immunity, complement components are able to orchestrate immune reactions by communicating with multiple immune cells (**Fig. 1**). In addition, complement contributes to the maintenance of homeostasis by recognizing and eliminating apoptotic and necrotic cells. Furthermore, through its activity, the solubility of circulating immune complexes is maintained, thereby facilitating their elimination[5–9].

As a first line of defense against pathogens, complement has to quickly detect, tag, destroy and eliminate microbes. This task can be accomplished only by a complex and tightly regulated assembly of proteins involving various soluble and surface-bound complement components, receptors and regulators (**Fig. 2**). Complement activation is usually initiated by the interaction of several pattern-recognition receptors with foreign surface structures. Depending on the activation trigger, the complement cascade follows one of three pathways: the classical, lectin or alternative pathway.

The recognition of antibody complexes on the surface of pathogens, mediated by the multimeric collectin C1q, was early recognized as initiator of an enzymatic cascade that was later termed classical pathway. In the related lectin pathway, complement activation is initiated by an interaction of mannose binding lectin (MBL) with high-density arrays of terminal mannose on bacterial surfaces. In both cases, these pattern-recognition proteins form complexes with serine proteases (C1r/C1s and MASP, respectively) that lead to the cleavage of C4 to its fragments C4b and C4a. After binding of C2 to C4b, the same protease complexes are responsible for generating the active C2a fragment, which is part of the classical pathway C3 convertase (C4bC2a; **Fig. 2a**). To guarantee instant response to pathogens, the cascade is constantly kept at a low level of

Samsung Bioepis Exhibit No. 1002
Page 986

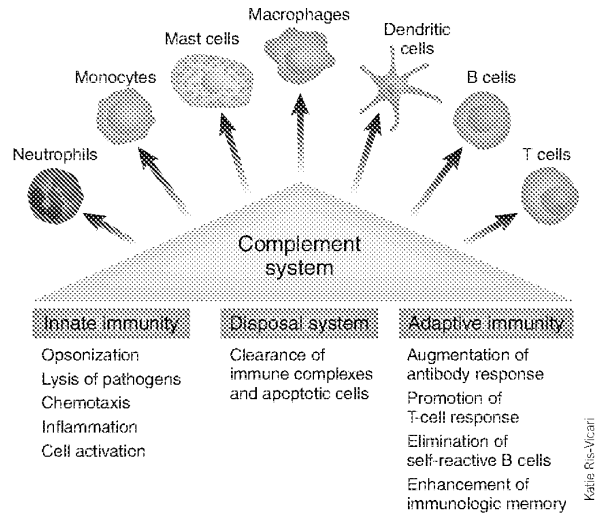


Figure 1 The complement system as a bridge between innate and adaptive immunity. Although the complement system has traditionally been considered part of the innate immune system, research in recent decades has revealed that complement is able to activate cells involved in both the adaptive and innate immune response. Complement triggers and modulates a variety of immune activities and acts as a linker between the two branches of the immune response. In addition, the complement system maintains cell homeostasis by eliminating cellular debris and immune complexes.

© 2007 Nature Publishing Group http://www.nature.com/naturebiotechnology

activity—'tick-over'—by the alternative pathway. Spontaneous hydrolysis of C3 to C3w induces a conformational change that enables binding to factor B and formation of the initial alternative pathway C3 convertase (C3wBb; **Fig. 2a**). The alternative pathway may represent up to 80% of complement activation in some instances and can also be triggered by various reactions with foreign surfaces.

All three pathways converge in the cleavage of C3 into its active fragments, C3a and C3b, by the C3 convertases. Formation of C3b exposes a previously hidden thioester group that covalently binds to patches of hydroxyl and amino groups on the pathogen surface. This process, termed opsonization, is critical for all subsequent steps in the complement cascade and the elimination of pathogens and apoptotic host cells by phagocytes. In an amplification loop, binding of factor B to surface-bound C3b and its cleavage by factor D generates the alternative pathway C3 convertase C3bBb, which causes a gradual acceleration of C3b production. Surface-tethered C3b also plays a central role in the formation of the two C5 convertases (C4bC2aC3b and C3bC3bBb), which cleave C5 into C5a and C5b. C5b initiates the assembly of the membrane attack complex (MAC), a pore that is formed by components C5b, C6, C7, C8 and multiple units of C9 and ultimately leads to cell lysis. The anaphylatoxins C3a and C5a, generated as a result of proteolytic cleavage of C3 and C5, respectively, are potent inflammatory mediators that trigger a plethora of processes, which culminate in the stimulation of immune cells and the elimination of the pathogen[5].

Despite years of intensive research in this field, our picture of complement activation seems to be far from complete. Very recently, additional activation pathways have been identified: for example, MBL-associated protease 2 (MASP-2) of the lectin pathway seems to directly attack and cleave C3 without formation of the corresponding C3 convertase (C2 bypass pathway; **Fig. 2a**)[10]. Similarly, direct cleavage of C3 and C5 by a series of extrinsic proteases such as kallikrein or thrombin (extrinsic protease pathway; **Fig. 2a**), has also been proposed. This activity points to an interesting connection to other essential plasma cascades such as the coagulation system[11]. Finally, properdin seems to not only stabilize the C3 convertase as a positive complement regulator but may also act as a pattern-recognition molecule and actively induce convertase formation on foreign surfaces[12,13].

One of the most fascinating aspects of complement is the sophisticated regulation mechanism that allows a rapid reaction to pathogenic intruders while protecting host cells from its destructive potential. After activation, the opsonization rates often reach several thousand C3b molecules per cell per minute. In principle, this attack is not specifically directed against foreign cells. However, interplay of time, location and molecule-based regulation mechanisms generally prevents the complement cascade from attacking host tissues. Both the thioester group of C3b and the C3 convertases have short half-lives, limiting their action to the site of activation. Whereas the convertase is actively stabilized on foreign cells by properdin (**Fig. 2a**), a set of soluble and membrane-bound negative regulators inactivates and degrades active molecules on host cells (**Fig. 2b**). These structurally related regulators of complement activation include factor H, decay accelerating factor (DAF/CD55), membrane cofactor protein (MCP/CD46), C4-binding protein (C4BP) and complement receptor 1 (CR1/CD35). These regulators either induce an accelerated decay of the convertases (DAF, CR1, factor H) or act as cofactors for factor I, which degrades C3b to its inactive form iC3b (MCP, CR1, factor H). An additional regulator of complement activation, CD59, prevents the formation of MAC by intercalating between C8 and C9 subunits (**Fig. 2b**)[5,14,15].

Complement's involvement in disease

The central role of complement in the orchestration of immune reactions inevitably renders it a target for immune evasion or attack by pathogens and also a contributor to many diseases. Even small disruptions in the delicate balance of activation and regulation can lead to an overreaction of complement that triggers inflammation and cell lysis. Many inflammatory, autoimmune, neurodegenerative and infectious diseases have been shown to be associated with excessive complement activity (**Fig. 3**). Complement involvement is usually complex and may include both an inappropriate initiation of the cascade and deficiencies in specific components or regulators[6,16,17].

Ischemia/reperfusion (I/R) injury is a severe consequence of conditions that lead to a deprivation and subsequent restoration of the tissues' blood supply; I/R injury can occur in a number of clinical conditions, including acute myocardial infarction, stroke, and hemorrhagic and septic shock, and may also be a complication of coronary artery bypass graft surgery. Paradoxically, it appears that the tissue reperfusion and not the ischemia activates complement and leads to inflammation-induced damage. Even though the exact involvement of complement activation in I/R injury is still unclear, several experimental studies have indicated a connection between complement and the pathogenesis of I/R injury, and have suggested complement inhibition as a potential therapy[18].

Complement seems to be a major contributor to a number of autoimmune diseases. For example, deposition of immune complexes during systemic lupus erythematosus initiates the classical pathway by binding of C1q (**Fig. 2b**) to these complexes[19]. A similar mechanism may also be involved in the pathogenesis of rheumatoid arthritis, and C5a (**Fig. 2b**) seems to have a major role in psoriasis and complement-mediated inflammatory diseases including asthma[20]. Complement activation by C1q, which acts as a pattern-recognition receptor for amyloidal plaques, has been correlated with the pathology of Alzheimer's disease[21]. Although complement also seems to be involved in other neurodegenerative diseases such as Huntington's disease or Parkinson's, the exact connection is less clear[21].

Samsung Bioepis Exhibit No. 1002
Page 987

© 2007 Nature Publishing Group http://www.nature.com/naturebiotechnology

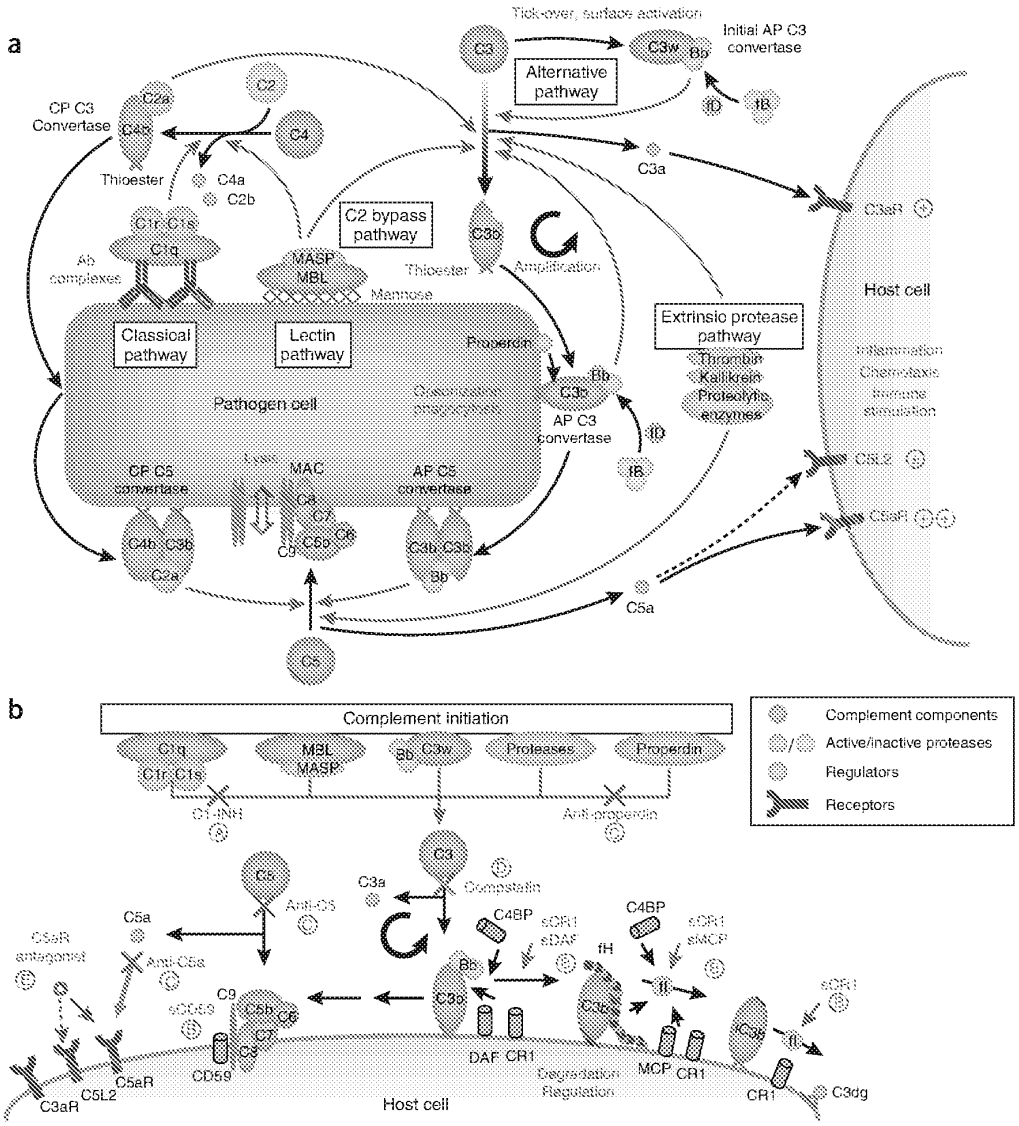


Figure 2 Activation, regulation and therapeutic modulation of the complement system. (**a**) The complement cascade after activation by pathogens. In addition to a low level of constant activation by the alternative pathway (AP; tick-over via hydrolyzed C3 (C3w) results in the formation of the initial C3 convertase C3wBb), the complement cascade is usually activated by antibody complexes (classical pathway) or high-density mannose (lectin pathway) on the surface of pathogens. This activation leads to the formation of the C3 convertases C4bC2a and C3wBb, which cleave native C3 to C3b and C3a. Deposition of C3b on cell surfaces via its thioester group initiates the cleavage of more C3 (the amplification loop via the final AP C3 convertase C3bBb), opsonization and phagocytosis, as well as lysis as a result of the formation of the membrane attack complex (MAC). In addition, the anaphylatoxins C3a and C5a are released and trigger further immune reactions upon binding to their receptors (C3aR, C5aR, C5L2). These combined actions of complement lead to the elimination of pathogenic cells. Recently, studies have shown that some steps of the cascade can be directly initiated by certain proteolytic enzymes (the extrinsic protease pathway) or by MBL/MASP (the C2 bypass pathway). Furthermore, the regulatory protein properdin may propagate and stabilize the formation of C3 convertases on the surface of the cell. Protein conversions are shown as black arrows and enzymatic reactions as green arrows. (**b**) Regulation, deactivation and inhibition of the complement cascade on host cells by natural regulators and complement-specific therapeutics. Several pathogenic processes and diseases are the result of an erroneous activation or insufficient downregulation of the complement cascade. Under normal conditions, any host-associated C3 convertase (C3bBb) undergoes an accelerated decay mediated by complement receptor 1 (CR1), decay accelerating factor (DAF), C4b-binding protein (C4BP) or factor H (fH). C3b is degraded to inactive iC3b by factor I in a reaction that requires as cofactor CR1, fH, C4BP or membrane cofactor protein (MCP). In addition, CD59 prevents the formation of the MAC. Some of the therapeutic interventions focus on increasing this downregulation by using soluble forms of these regulators (that is, sCR1, sDAF, sMCP, sCD59). Other approaches involve the substitution of the natural C1 inhibitor (C1-INH), the inhibition of the central conversion of C3 to C3b and C3a (compstatin), blockage of C5 or C5a by antibodies, and the suppression of anaphylatoxic signaling by C5a receptor antagonists. For clarity, only the regulation of the alternative pathway, which may contribute up to ~80% of all complement activity, is shown here. The C3 convertase of the classical pathway (C4bC2a), as well as the C5 convertases (C4bC2aC3b and C3bC3bBb), can be modulated by the same regulators and drug compounds. Therapeutic modulators in clinical trials or late preclinical development are indicated in red, with the circled letter referring to the corresponding drug class in **Tables 1** and **2** and the main text.

Samsung Bioepis Exhibit No. 1002
Page 988

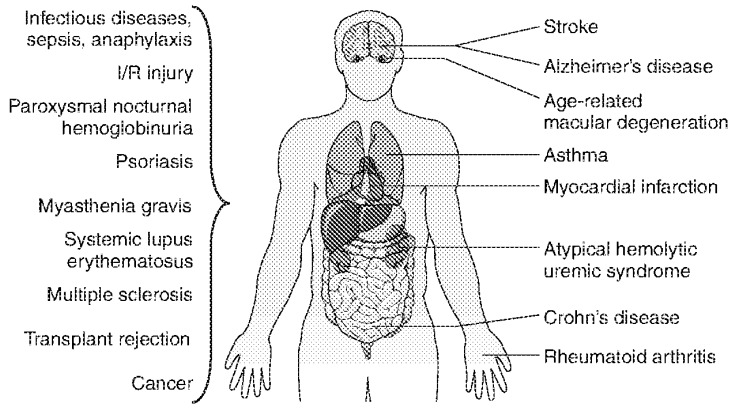


Figure 3 Examples of pathological conditions involving the complement system. Erroneous activation or insufficient regulation of the complement cascade may lead to an attack by the immune system against self-tissue. Many autoimmune, inflammatory and ischemia/reperfusion (I/R) injury-related diseases are therefore connected with complement. Whereas some of these pathological conditions are localized to specific organs and tissues, many of them are systemic. In addition, some pathogens have found ways to evade or even misuse the complement system, thereby contributing to infectious diseases and their consequences.

© 2007 Nature Publishing Group http://www.nature.com/naturebiotechnology

Alterations in the expression and functions of complement regulators may be another cause of complement-related diseases. For example, polymorphism and mutations in genes encoding factor H (**Fig. 2b**) are associated with age-related macular degeneration (AMD), the major cause of blindness in industrial nations, and atypical hemolytic uremic syndrome (aHUS), respectively[22]. Low plasma levels of C1 inhibitor have been found to be responsible for hereditary angioedema (HAE)[23,24], and a lack of CD59 (**Fig. 2b**) on erythrocyte surfaces causes paroxysmal nocturnal hemoglobinuria (PNH)[25].

Finally, a series of pathogens have found ways to evade or even hijack complement proteins. Microbial proteins that either inhibit complement components or mimic regulators have been identified in both viruses (e.g., HIV, smallpox, Epstein-Barr virus) and bacteria (e.g., *Staphylococcus aureus, Streptococcus pneumoniae*)[5,26–30].

Druggability of complement

The central role of complement in the pathophysiology of major diseases makes it an interesting target for the pharmaceutical industry. However, despite numerous attempts in recent years to inhibit or modulate complement therapeutically[17,31–34], the success rate has been disappointingly low. Before the recent approval of complement-specific drugs, many promising candidates encountered insuperable obstacles while in clinical development. Inevitably, this high attrition rate raises questions about what renders complement drug discovery such a difficult task.

The multifaceted nature of both the cascade and its disease involvement may be one central problem. Many questions about the exact disease-related mechanisms of complement, both at the molecular and clinical level, are still unresolved, and this lack of clarity complicates specific targeting. "Druggability" (**Box 1**) is another major issue. Traditional drug discovery aims for small molecular entities with the potential for oral administration, yet many critical steps in the complement cascade are fundamentally based on large protein-protein interactions, which are challenging to influence with small molecules[35]. Anaphylatoxin receptors and serine proteases are the only complement targets with an auspicious druggability potential. This limitation may also explain the exceptionally high number of biopharmaceuticals (proteins, antibodies, peptides, nucleotides) and biotechnology startups that are involved in the current drug development process (**Tables 1** and **2**). Finally, the optimal target within the complement cascade (**Fig. 2b**) and the extent of inhibition has to be critically defined for each indication. Suppressing pathogenic complement activity without compromising its defensive and immunomodulatory functions might be one of the most delicate challenges when treating chronic conditions. Intervention at the activation level may require a highly specific approach, for example, inhibiting the recognition of amyloid plaques by C1q in the case of Alzheimer's disease. However, inhibition of a single pathway may be insufficient for many diseases and can still put pathogen defense at risk. Inhibition of C3 and its convertase potentially has the broadest effect, because it blocks all terminal activity of complement as well as the amplification loop. However, this nearly complete blockage may also lead to adverse effects and therefore favors local or targeted applications. Prevention of terminal complement action, for example, by inhibiting C5a formation and binding, is highly specific but requires the most information about the target involved. Finally, supplementation or inhibition of complement regulators may offer a more physiologic and specific way to modulate complement activity, especially in the treatment of chronic diseases.

Current complement therapeutics

A number of promising approaches for the clinical substitution, inhibition or modulation of complement have been developed and are presented in the following sections. We have classified complement therapeutics that either have been in clinical trials (**Table 1**) or are in the late steps of preclinical development (**Table 2**) into five major categories according to the mechanism of action (labeled **a–e** in **Tables 1** and **2**, and **Fig. 2b**).

Serine protease inhibitors. The consecutive cleavage and activation of several proteases constitutes the driving force behind complement function. Eight serine proteases are integral elements of the complement cascade itself (C1r, C1s, C2a, MASP-1, MASP-2, factor D, factor B, factor I)[36], whereas others are involved through the extrinsic protease pathway (**Fig. 2**)[11]. Because proteases are among the most druggable targets in the complement system (**Box 1**), it is not surprising that early drug development efforts focused on protease inhibitors[37]. Although screening- and structure-based approaches resulted in drug candidates for factor D (BCX-1470, BioCryst, Birmingham, AL, USA)[38] and C1s (C1s-INH-248, Knoll/Abbott, Abbott Park, IL, USA)[39], short half-lives and a lack of specificity have thus far prevented the successful development of small-molecule protease inhibitors for the treatment of complement-related disorders.

The only complement-associated protease inhibitor currently on the market is C1-inhibitor (C1-INH), a heavily glycosylated plasma protein that is used in the treatment of HAE[23,24,40,41]. This disease is characterized by recurrent episodes of severe skin and mucous membrane edema and is caused by either an insufficient production of C1-INH (type 1) or a mutation around its binding site that leads to inactivity (type 2). In both types, therapeutic supplementation of this protein has proven to be an effective and safe treatment for HAE and helps prevent severe disease

Samsung Bioepis Exhibit No. 1002
Page 989

relapses and life-threatening complications[42,43]. A third HAE type of unknown etiology and low prevalence does not benefit from supplementation, because it is not dependent on C1-INH activity[44]. Although C1-INH gained its name from its ability to block the serine esterase activities of C1r and C1s[45], its specificity includes other proteases, such as MASP, kallikrein and coagulation factors XI and XII. Although plasma levels of C2 and C4 are clearly affected by HAE, the primary mechanism of C1-INH seems to be more closely associated with bradykinin-kallikrein than with the complement system[23]. However, there is increasing evidence that therapeutic administration of C1-INH may also be beneficial in other disorders with a direct connection to complement, such as I/R injury[41,46–48].

Whereas pasteurized C1-INH concentrates for the treatment of HAE have been available since 1985, a lack of licensed products still limits the accessibility of this treatment in several countries, including the United States[43]. Fortunately, annotation of orphan drug status by the FDA (**Box 2**) and licensing agreements with established providers have boosted the development and registration of this important drug, and its approval in the United States is imminent[49]. Current C1-INH formulations are still being improved[50], for example, by the use of nanofiltered concentrates that feature better safety with regard to viral contamination. The high production costs and extensive quality control requirements for plasma-derived C1-INH still makes it an expensive treatment option and will certainly limit its availability in poorer countries. Development of a recombinant form, which is isolated as a glycosylated protein from the milk of transgenic animals, may be an important step in overcoming these limitations[51]. Experience with C1-INH in the clinic and its good safety profile are expected to lead to an extension of its indications. For example, clinical trials regarding the use of C1-INH for the prevention of I/R injury after acute myocardial infarction are currently planned.

Table 1 Complement therapeutics on the market or in clinical trials

Product (company)	Activity	Stage of development
Protease inhibitors (A)		
C1-INH (Cetor/Sanquin, BerinertP/CSL Behring, Lev Pharma)	Purified plasma protein; inhibition of C1r/C1s, kallikrein and other proteases	Marketed (HAE)[a]; clinical phase 3 (HAE)[a]; preclinical/phase 1 for other indications (AMI, CABG)
Rhucin/rhC1INH (Pharming Group N.V.)	Recombinant human C1-INH from transgenic rabbits	Clinical phase 3 (HAE)
Soluble complement regulators (B)		
sCR1/TP10 (Avant Immunotherapeutics)	Extracellular part of CR1; decay accelerator, factor I cofactor	Clinical phase 2 (CABG)[b]
CAB-2/MLN-2222 (Millenium Pharmaceuticals)	Chimera of DAF and MCP; decay accelerator, factor I cofactor	Clinical phase 1 (CABG)[b]
Therapeutic antibodies (C)		
Eculizumab/ Soliris (Alexion Pharmaceuticals)	Humanized long-acting mAb against C5	Marketed (PNH), preclinical for other indications
Pexelizumab (Alexion Pharmaceuticals)	Humanized short-acting mAb against C5	Clinical phase 3 (AMI, CABG)[b]
Ofatumumab (Genmab A/S)	Humanized anti-CD20 mAb	Clinical phase 2
Complement component inhibitors (D)		
Compstatin/POT-4 (Potentia Pharmaceuticals)	Peptidic C3 inhibitor; no generation of C3a/C3b	Clinical phase 1 (AMD)
Receptor antagonists (E)		
PMX-53 (Peptech Ltd.)	Peptidic C5aR antagonist	Clinical phase 2 (RA, psoriasis)
Other		
rhMBL (Enzon Pharmaceuticals)	Recombinant human MBL as substitution therapy	Clinical phase 1b (MBL deficiency)

[a]C1-INH preparation are only approved in some countries, whereas they are in clinical phase 3 in the USA. [b]Clinical trials have recently been performed but no product development has been undertaken since then, or the development was officially discontinued. AMD, age-related macular degeneration; AMI, acute myocardial infarction; CABG, coronary artery bypass grafting; HAE, hereditary angioedema; PNH, paroxysmal nocturnal hemoglobinuria; RA, rheumatoid arthritis.

Soluble complement regulators. Because regulators of complement activation are natural modifiers of complement activities and prevent a host cell from being attacked by its own defense system, they have been considered for therapeutic use since the early stages of complement drug discovery[52]. A first breakthrough was reached with the expression of a soluble form of complement receptor 1 (sCR1)[53]. This molecule featured both decay accelerator and cofactor activity and had a high potency in inhibiting both the classical and alternative pathways. sCR1 showed promising results in the treatment of I/R injury and various other conditions in experimental animal models. Based on these encouraging results, sCR1 was developed as a therapeutic (TP10; Avant Immunotherapeutics, Needham, MA, USA) for use after coronary artery bypass graft surgery. TP10 is expressed as a 240-kDa glycoprotein in Chinese hamster ovary cells, has a plasma half-life of ~55 hours and is safe and well-tolerated in both adult and infant patients[54]. In a large placebo-controlled phase 2 trial, comprising 564 high-risk patients undergoing cardiac surgery, a single intravenous bolus of TP10 immediately before surgery was found to inhibit complement activation for up to 3 days postoperatively. Whereas TP10 treatment resulted in a significant improvement of the clinical endpoints in males, no such benefits were observed in female participants. This surprising gender specificity was confirmed by a subsequent phase 2b study comprising 297 women. Whereas the mechanism governing these differences is still unclear, effects other than complement inhibition are likely to be involved. As a consequence, a male-only indication of TP10 for bypass surgery was considered for clinical phase 3. TP10 also showed promising results in smaller studies of adults undergoing lung transplants and infants undergoing coronary artery bypass graft surgery[54]. However, Avant Immune has recently decided not to continue investing in clinical trials of TP10 (ref. 55). It remains to be seen whether this development will mean the end of sCR1 as a therapeutic compound. Improvements in the structure (e.g., a reduction of its size) and formulation of sCR1 or an extension of its indications may well lead to its resurrection. Although the first steps in this direction have already been taken, neither TP20 (sCR1 with sialyl Lewis^X-glycosylation for targeting sites of inflammation[56], Avant Immune) nor Mirococept/APT070 (a truncated sCR1 with a membrane-tethering motif[57], Inflazyme Pharmaceuticals, Richmond, BC, Canada) has been tested in clinical trials.

Soluble forms of MCP, DAF and CD59 have also been considered as therapeutics. Whereas DAF and MCP each offer only a single regulatory activity, a recombinant chimera of their extracellular parts has been developed[17]. The resulting sDAF-sMCP hybrid was initially named "complement activity blocker 2" (CAB-2; Xoma, Berkeley, CA, USA) but entered clinical trials with a new name and licensing partner (MLN-2222; Millennium, Cambridge, MA, USA). However, no further studies have been initiated recently. Like Mirococept/APT070, a membrane-tethering sCD59 has been developed by Inflazyme Pharmaceuticals. Although

© 2007 Nature Publishing Group http://www.nature.com/naturebiotechnology

Samsung Bioepis Exhibit No. 1002
Page 990

Table 2 Complement therapeutics in pre-clinical development

Product (company)	Activity	Stage of development
Protease inhibitors (A)		
Factor D inhibitors[a] (BCX1470 etc.)	Small molecule serine protease inhibitors	Mostly discontinued (due to lack of specificity and/or short half-life)
Soluble complement regulators (B)		
sCR1-sLe^x/TP-20 (Avant Immunotherapeutics)	sCR1 with sLe^x-rich glycosylation to target sites of inflammation	Development/preclinical (stroke, heart attack)
Mirococept (Inflazyme Pharmaceuticals)	sCR1 with lipopeptide membrane linker	Preclinical (AMI, inflammatory diseases)
Therapeutic antibodies (C)		
TNX-234 (Tanox)	Humanized antibody against factor D	Preclinical (wet AMD)
TNX-558 (Tanox)	Humanized antibody against C5a	Development/preclinical (inflammatory diseases)
TA106 (Taligen Therapeutics)	Antibody against factor B	Development/preclinical
Neutrazumab (G2 Therapies)	Antibody blocking the C5a receptor	Development/preclinical (RA, stroke)
Anti-properdin (Novelmed Therapeutics)	Antibody against properdin	Development/preclinical
HuMax-CD38 (Genmab A/S)	Humanized anti-CD38 mAb that triggers complement	Preclinical studies (cancer, multiple myeloma)
Complement component inhibitors (D)		
ARC1905 (Archemix)	Aptamer-based C5 inhibitor (PEG-ylated)	Preclinical (AMD)
Receptor antagonists (E)		
JPE-1375, JSM-7717 (Jerini[b])	Small molecule/peptidomimetic antagonists for C5a receptor	Preclinical (inflammation, renal and ocular diseases[b])

[a]Currently, there are no complement-specific serine protease inhibitors listed in the pipelines of pharmaceutical companies. [b]Ophthalmology compounds are developed further by Jerini Ophthalmic.

© 2007 Nature Publishing Group http://www.nature.com/naturebiotechnology

not listed in the company's pipeline, experimental studies were recently performed to investigate its potential therapeutic usefulness as a treatment option for PNH[58]. This rare, genetic, life-threatening blood disorder leads to decreased expression of membrane-anchored proteins, including CD59 and DAF, on erythrocytes. Substitution and membrane tethering of recombinant CD59 may develop into a promising therapy for PNH[58].

Therapeutic antibodies. Following a general trend in the pharmaceutical industry, antibody-based therapeutics appear to be the most rapidly growing drug class against complement-related diseases. Crucial developments in their screening, production and humanization have led to a remarkable boost in the number of therapeutic antibodies[59]. By targeting specific components of the complement system, all stages from the initiation and activation process to single terminal actions can hypothetically be blocked in a selective manner. Current drug candidates in the pipeline focus primarily on the inhibition of downstream processes around C5 and its cleavage fragment, the anaphylatoxin C5a.

Selective inhibition of C5 using monoclonal antibodies (mAb) has been considered a promising therapeutic option for many years. A highly selective mAb against mouse C5 was introduced by Frei *et al.* two decades ago[60] and was later demonstrated to be effective in a mouse model of rheumatoid arthritis by Alexion Pharmaceuticals[61]. Continuous improvement and clinical testing finally led to the FDA approval of eculizumab[3], which is currently the only complement-specific antibody on the market[62]. Eculizumab is the first and only approved therapy for PNH, a rare but life-threatening disorder that is characterized by a chronic destruction of red blood cells. A mutation on the X chromosomes of hematopoietic stem cells prevents the proper biosynthesis of the glycosylphosphatidylinositol (GPI) anchor, which leads to a deficiency in membrane-anchored proteins, including DAF and CD59. The lack of efficient inhibition of complement activation on red blood cells results in an increase in MAC formation on erythrocyte membranes and cell lysis. The survival of PNH erythrocytes is dramatically reduced in PNH patients, to 10% of normal red blood cells. On platelets, the absence of CD59 and increased MAC formation can lead to morphological changes of their surface. These platelets show enhanced susceptibility for activation, contributing to a high risk of thrombosis in PNH patients. Until very recently, only supportive therapies (blood transfusions, iron therapy, anticoagulation and others) or allogeneic stem cell transplantation had been available for the clinical management of this disease[25].

Given the complement-focused molecular mechanism of PNH, prevention of MAC activity evolved as the most promising treatment strategy. Two possible approaches were identified: substitution of defective CD59 or neutralization of complement proteins involved in MAC formation itself, as in the case of eculizumab. By tightly binding to C5 and preventing its cleavage to C5b, this new drug suppresses the formation of the MAC and reduces the clinical symptoms of PNH. Eculizumab (5G1.1-SC) is a humanized version of the anti-C5 antibody h5G1.1, which was first described in 1996 (ref. 63). Eculizumab received orphan drug status for PNH from both the FDA and the EU in 2003, and Alexion Pharmaceuticals subsequently initiated two phase 3 trials. In both studies, eculizumab was safe and well-tolerated, and no development of antibodies against the drug was detected. Because complement inhibition is expected to lead to a higher susceptibility to infection, particularly with *Neisseria meningitidis*, all patients had been vaccinated with a meningococcal vaccine before beginning treatment. The antibody was clearly effective: 51% of those who received eculizumab gained independence from blood transfusions (versus 0% in the placebo group). Both the endogenous erythrocyte mass and the hemoglobin levels were remarkably higher during treatment than before, and many symptoms related to quality of life (e.g., fatigue, abdominal pain) were attenuated. Whereas the preliminary data also indicated a lower risk for thrombosis, this benefit needs to be confirmed by ongoing clinical trials[25,64,65].

As an anti-C5 antibody that inhibits the generation of both C5b and the anaphylatoxin C5a, the potential indications for eculizumab are certainly not limited to PNH. Consequently, eculizumab has undergone several preclinical and clinical studies for a variety of conditions (e.g., psoriasis, rheumatoid arthritis, systemic lupus erythematosus and transplant rejection). Even though some of these paths seem to have been discontinued[65], Alexion Pharmaceuticals still performs preclinical development of eculizumab for autoimmune diseases and transplant rejection. Furthermore, nebulized eculizumab for the treatment of asthma and an intravitreal application to treat age-related macular degeneration are in the company's pipeline. In the case of pexelizumab, the short-acting sc-Fv fragment of eculizumab, several phase 3 trials have been done to test its potential for use in coronary artery bypass graft surgery and acute

Samsung Bioepis Exhibit No. 1002
Page 991

myocardial infarction[66]. Despite promising preclinical results, however, data from these clinical trials did not show significant improvement in the treatment group when compared to the placebo group[67,68]. Alexion Pharmaceuticals has therefore announced that it plans to focus its development efforts on eculizumab[69].

The concept of using humanized antibodies (and their fragments) to inhibit certain steps of the complement cascade seems to be a very productive strategy. Several companies list anticomplement antibodies in their pipelines (**Table 2**). Two of them target the various actions of the anaphylatoxin C5a. Neutrazumab (G2 Therapies, Darlinghurst, NSW, Australia) binds to extracellular loops of C5aR and thereby inhibits the binding of C5a to its major signaling receptor. G2 Therapies recently announced a partnership with Novo Nordisk (Bagsværd, Denmark) with regard to the development of their anti-C5aR antibodies. Another approach has been taken by Tanox (now part of Genentech, S. San Francisco, CA, USA) with their antibody TNX-558, which neutralizes C5a by binding to the anaphylatoxin itself. Interestingly, TNX-558 binds to a C5a epitope in native C5 but does not prevent its cleavage. Tanox has also developed an antibody directed against factor D (TNX-234). Together with the anti–factor B (TA106 for age-related macular degeneration and asthma[70]; Taligen, Aurora, CO, USA) and anti-properdin (Novelmed, Cleveland, OH, USA) antibodies in development, these biologics may offer additional approaches for inhibiting complement at the C3 convertase level.

Whereas inhibition of complement activity is the desired outcome in the vast majority of therapeutic approaches, its local stimulation may be beneficial in some malignant diseases, such as cancer. Experience with monoclonal antibodies in cancer therapy suggests that induction of cell death through antibody-dependent cellular cytotoxicity or complement-dependent cytotoxicity (CDC) may be a major driving force behind their effectiveness (e.g., in the case of the anti-CD20 mAb rituximab from Roche). However, relatively high antigen densities are required for an efficient initiation of the classical pathway by C1q. Genmab (Copenhagen) recently described anti-CD20 antibodies with exceptionally high CDC potency[71,72]. Both the location of the epitope as well as the dissociation rate are apparently responsible for this high level of effectiveness. Ofatumumab/HuMax-CD20 has been selected for clinical development and has recently been evaluated in terms of its effectiveness in treating acute rheumatoid arthritis (phase 2), B-cell chronic lymphocytic leukemia (phase 3) and follicular lymphoma (phase 3). Other antibodies with high CDC potency against multiple myeloma (HuMax-CD38) or colon, pancreatic and prostate cancer (HuMax-ZP3) are currently in preclinical development (http://www.genmab.com/). This strategy is a good example of how to make use of a more general, nonspecific property and select and optimize it to obtain the desired clinical effect. Because CDC may generally be induced by therapeutic IgGs (and IgMs), the CDC activity of these reagents may become a crucial parameter in the development of therapeutic antibodies.

Complement component inhibitors. Although antibodies may be considered the archetype of site-blocking compounds, smaller molecules such as peptides, nucleotides and synthetic molecules may also have the potential to interrupt protein functions by steric hindrance or the induction of conformational changes. Small functional inhibitors of complement activity are expected to have drug-like properties with enhanced pharmacokinetic profiles (**Box 1**). These advantages may make them suitable for drug development efforts related to oral bioavailability and better administration routes.

Compstatin is the most developed candidate in this class of substances and recently entered clinical trials. This cyclic tridecapeptide has been discovered through screening of combinatorial phage-display libraries[73] in the research group led by one of the authors of this review. It effectively prevents the cleavage of C3 to its active fragments C3a and C3b and therefore inhibits the most central step in the complement cascade. Although the exact mechanism of compstatin's inhibitory action has not yet been resolved, the recent publication of the cocrystal structure between compstatin and C3c suggests that a disruption of protein-protein interactions during convertase formation may be an important determinant[74]. This finding has led to the hypothesis that a conformational change or an interruption in protein-protein interactions is responsible for its activity. Compstatin has shown effective complement inhibition in a variety of experimental disease models[34]. Despite a narrow selectivity for primate C3 that may affect preclinical animal experiments[75], its high efficacy and rather small size make it a promising drug candidate. Its inhibitory efficacy has been increased by 264-fold[34,76] through the use of rational and combinatorial synthesis, structural analysis, and computational approaches to identify active analogs. Potentia (Louisville, KY, USA) has announced the inauguration of clinical trials for the treatment of neovascular age-related macular degeneration by intravitreal application of a compstatin analog (POT-4).

Combinatorial approaches have also been used to identify a short aptamer molecule directed against C5 (ref. 78). Aptamers are single-stranded nucleotide stretches that have molecular recognition properties similar to those of antibodies, but offer better options for derivatization. They can be selected in an automated high-throughput process known as systematic evolution of ligands by exponential enrichment (SELEX)[79]. More than 15 years after the initial description of this method, the first aptamer-based drug pegaptanib (Macugen; Eyetech Pharmaceuticals/Pfizer, New York) a PEG-ylated anti–vascular endothelial growth factor (VEGF) aptamer for the treatment of wet age-related macular degeneration, was approved by the FDA[80]. The optimized and heavily modified

Box 1 Drugs and druggability

For the pharmaceutical industry, the ideal drug is a small molecule featuring high potency, selectivity, few adverse effects, low production costs and administration in a single daily oral dose. However, identifying compounds with these properties is challenging, and drug companies have been confronted with an ever-growing drug attrition rate[97]. During the drug discovery process, molecules that fulfill a set of molecular guidelines for oral bioavailability, known as the "Rule of 5"[98] (RO5; e.g., a molecular weight below 500 Da or a partition coefficient [logP] below 5), are referred to as "drug-like" compounds. Similarly, this concept can be transferred to the targets of therapeutic intervention. Druggability refers to disease-related proteins that have the potential to be modulated by drug-like compounds[97]. Several attempts have been made to predict the druggability of protein families and to identify promising drug targets. Using the "druggable genome" approach, the targets of currently marketed drugs have been analyzed and mapped to protein families, assuming persistent druggability within the family[99]. Enzymes are the predominant class (nearly 50% of the total), followed by G-protein-coupled receptors (30%) and ion channels (7%). Within the complement cascade, the only targets with acceptable druggability potential are therefore the serine proteases and the anaphylatoxin receptors. Considering the large post-translational variability of protein targets and the increasing market for biopharmaceuticals, expansions of this model, such as the "pharmaceutically tractable genome," have recently been suggested[100].

© 2007 Nature Publishing Group http://www.nature.com/naturebiotechnology

Samsung Bioepis Exhibit No. 1002
Page 992

© 2007 Nature Publishing Group http://www.nature.com/naturebiotechnology

Box 2 Supporting the niches—the Orphan Drug Act

Development of a new drug compound is a challenging and expensive endeavor. The cost for a newly marketed drug is currently estimated to be in the range of $500 million to more than $2,000 million[101]. It is therefore understandable that pharmaceutical companies focus primarily on drugs to treat major diseases with a large market potential (that is, "blockbuster drugs"). This focus often leaves a painful therapeutic gap for rare diseases, niche markets or biopharmaceuticals with patent limitations (e.g., natural proteins). Compounds that are unprofitable to develop are usually referred to as "orphan drugs"[102]. In an effort to encourage the development of such drugs, several drug regulation authorities, including the US Food and Drug Administration (FDA), have initiated special programs such as the Orphan Drug Act (ODA) of 1983 (ref. 103). Designation of orphan drug status is coupled to a series of financial incentives, the most important of which is marketing exclusivity for 7 years after drug approval. In addition, orphan product development grants sponsor clinical trials and close the gap between basic research and clinical development. Undeniably, the ODA is one of the most successful recent United States legislative actions and has had a major impact on the drug market. Twenty years after its enactment, over 1,000 drugs had been accorded orphan drug status, and more than 200 had been approved for marketing, thereby improving the clinical situation of an estimated 11 million patients in the United States alone[102]. Meanwhile, these numbers have grown to 1,750 designated and 315 marketed orphan drugs (as of October 4, 2007, http://www.fda.gov/orphan/designat/list.htm). Similar initiatives also exist in other countries and regions (e.g., at the European Medicines Agency). In the complement field, eculizumab, for the treatment of paroxysmal nocturnal hemoglobinuria, and C1 inhibitor concentrate, for treatment of hereditary angioedema, are covered by the ODA. For more information, see http://www.fda.gov/orphan/.

form of the anti-C5 RNA aptamer (ARC1905; Archemix, Cambridge, MA, USA) features a subnanomolar binding affinity for human C5. It provides complete blockage of downstream complement activation by inhibiting the cleavage of C5 into C5a and C5b. A 40-kDa PEG group has been added to the 5′ end to improve its pharmacokinetic properties. Although ARC1905 was initially developed for use during coronary artery bypass graft surgery, the recent failure of pexelizumab for this indication has had a direct impact on the fate of ARC1905. Like compstatin (POT-4) and Macugen, the compound is now positioned as an intravitreal treatment option for wet and dry age-related macular degeneration and is awaiting clinical trials (P. Bouchard, Archemix Corp., personal communication).

Anaphylatoxin receptor antagonists. The proinflammatory activity of the anaphylatoxin C5a is the driving force behind many complement-associated disorders. After its liberation from C5, this hormone-like glycoprotein binds to two high-affinity receptors, the C5a receptor (C5aR) and C5L2 (**Fig. 2**)[81]. Most of the proinflammatory signaling seems to be induced by C5aR, a G-protein-coupled receptor that is expressed on numerous myeloid (e.g., neutrophils, macrophages) and nonmyeloid cells. Despite its similar structure and affinity for C5a, the C5L2 receptor produces a completely different signaling pattern. In plasma, the activity of C5a is controlled through the removal of the C-terminal arginyl residue by the plasma enzyme carboxypeptidase N. The resulting $C5a^{desArg}$ binds poorly to C5aR but with a high affinity to C5L2. Earlier reports about binding of C3a and $C3a^{desArg}$ to C5L2 have recently been questioned and require further investigation[81]. The differential binding pattern, as well as reports of the anti-inflammatory activity mediated by C5L2, has led to the hypothesis that the regulation of C5a involves a sophisticated feedback mechanism[20,64]. However, other studies have reported a proinflammatory or C5aR-supporting role for C5L2 (ref. 82), leaving its exact function a matter of scientific debate.

Selective inhibition of the binding of C5a to its receptors offers a very promising opportunity for dampening the inflammatory response without depleting the defensive potential of complement[64,83,84]. The macromolecular biopharmaceuticals we have previously discussed either inhibit C5a generation (anti-C5 antibodies and aptamers) or neutralize the C5a-C5aR interaction by shielding the relevant binding site. C5aR antagonists, on the other hand, are designed to bind to the receptor with high affinity without inducing any signaling activity (**Fig. 2b**). Because only one of the two proposed binding sites on C5aR has to be targeted for this purpose[81,84], these antagonists are expected to be rather small. Simultaneous efforts by many companies have focused on reducing the functional fragment of C5a to a C-terminal hexapeptide, while replacing individual residues to shift its activity from agonistic to antagonistic. Cyclization of these hexapeptides not only allows them to be locked into the desired turn-conformation but also gives them higher stability with regard to proteolytic degradation[84].

The most promising candidate to emerge, the cyclic peptidomimetic PMX-53, is currently under development by Peptech[85]. With a molecular size under 1 kDa and a high oral bioavailability, PMX-53 has many of the desired features of a designed drug. However, its short half-life of only 70 min may mean a requirement for frequent, if not continuous, administration. Although the drug showed high stability in blood, gastric and intestinal contents, it was rapidly degraded by proteolysis in intestinal mucosa washings. In a phase 1a/2b trial in 21 patients with rheumatoid arthritis, PMX-53 was orally administered and showed positive trends in many disease measures. An equally positive result was observed in a phase 1b/2a trial of topical application of the drug in ten people with psoriasis. In both studies, PMX-53 was found to be safe and well-tolerated[85]. Despite these encouraging results, the short half-life and rapid degradation in intestinal mucosa of this drug may severely limit its use. It remains to be seen if Peptech will further develop PMX-53 or initiate new trials with PMX-205, a similar yet more hydrophobic compound that shows high potency but a much lower susceptibility to proteolysis than that of PMX-53 (refs. 86,87).

Given the current uncertainty about the role of the C5L2 receptor, a critical evaluation of the binding specificity may be an important requirement for the development of successful C5aR antagonists. Several companies seem to have promising candidates in preclinical testing. For example, Jerini has a series of peptidomimetics and small organic molecules (JPE-1375, JSM-7717) for the treatment of inflammatory, renal and ocular diseases in preclinical stages (http://www.jerini.com/).

Other concepts. Whereas most of the previously discussed concepts are designed to inhibit or decrease complement activation, one compound is intended to boost low complement activity. Approximately 30% of the human population shows low plasma levels of MBL, making them more susceptible to infections under immunosuppressive conditions. Recombinant human MBL (rhMBL; Enzon, Bridgewater NJ, USA) restores the activity of the lectin pathway and has been found to be safe and well tolerated[88]. It is currently being tested in phase 1b trials as replacement therapy in people with multiple myeloma who are deficient

Samsung Bioepis Exhibit No. 1002
Page 993

in MBL and undergoing high-dose chemotherapy and stem cell transplants, and in MBL-deficient people undergoing liver transplantation. Additional approaches such as quenching of exposed thioester groups of C3b (thioester inhibitors)[17] or inhibition of the C1q-IgG interaction by anionic small molecules (e.g., steroid/triterpenoid sulfates) have been described[31,89] but have not appeared in preclinical settings thus far.

Perspectives and outlook

As is true for complement research itself, the quest for complement-directed therapeutics has made dramatic changes in direction and encountered difficult challenges; initial euphoria has given way to a more realistic view of strategies and aims. Whereas some first-generation approaches of complement drug discovery have either failed (e.g., small-molecule serine protease inhibitors) or are severely struggling (e.g., soluble regulators), there are many promising concepts on the horizon that represent a second generation of complement drugs. Current indications for complement drugs, such as HAE or PNH, certainly represent niche markets far away from blockbuster areas. Both eculizumab and the C1-INH concentrates are protected under the Orphan Drug Act (**Box 2**), and their approval is an important step in establishing the concept and accumulating clinical experience before expanding their indications.

Undeniably, the unsatisfactory results obtained for TP10 and pexelizumab have left a bitter taste and may have had a negative impact on the field. These disappointments underscore the limitations inherent in broadly applying complement-specific drugs in inflammatory diseases. Many of the compounds that have been discontinued were designed to target multifaceted disorders such as I/R injury. In these disorders, many parallel mechanisms are involved, and the influence of complement is still a matter of debate. For such complex indications, the key to success may be to use complement drugs not as single therapies but (in consonance with their name) as complementary therapies. Clearly, more basic research and additional tests are required in this context. In cases in which clear complement targets can be identified (as in HAE or PNH), complement therapeutics have already been successfully applied.

Several factors are expected to boost the development of complement therapeutics. In particular, our accumulating knowledge about the exact role of complement in various diseases will greatly facilitate the identification of relevant targets. Even more importantly, crystal structures of a number of crucial complement components (e.g., C3 (ref. 90), C3b[91,92], C3c[90], C3d[93], factor B[94], factor D[95]) have recently been solved and are available for structure-based design. Genentech has reported a co-crystal structure of C3b and C3c with the extracellular domain of the macrophage complement receptor CRIg[92]. The same group recently demonstrated that soluble murine CRIg selectively inhibits the alternative pathway by competing with C5 for convertase binding, and that it was effective in mouse models of arthritis[96]. Its high selectivity for the alternative pathway and the availability of a detailed crystal structure make CRIg an attractive lead structure and render its further clinical development highly likely. Along with the continuous development of established approaches, the quest for complement therapeutics may get help from an unexpected source: human pathogens. *Staphylococcus aureus* (*S. aureus*) alone offers several interesting candidates. Among them, the extracellular fibrinogen-binding protein[28] and the staphylococcal complement inhibitor[30] of *S. aureus* have been reported to inhibit the conversion of C3 to C3a and C3b. An understanding of their interaction sites and mechanisms could be applied to developing either potent complement inhibitors or new antibiotics targeting *S. aureus*. In addition, the chemotaxis inhibitory protein of *S. aureus*[29] has been found to antagonize C5aR and may therefore serve as a lead structure for novel antagonists.

In many aspects, complement drug discovery illustrates general trends that are observed in the pharmaceutical industry. Limitations in the development of small compounds (e.g., lack of specificity or inability to disrupt protein-protein interactions) and improved technology and safety in the production of natural macromolecules has led to a growing market for these biopharmaceuticals. An analysis of the compounds indicates that they almost exclusively represent large proteins and nucleotides, or that they have been developed from peptides. Furthermore, major pharmaceutical companies tend to outsource early drug discovery phases to academic institutions and small startup companies. Partnerships with major pharmaceutical companies are usually only sealed after clinical trials have begun (often after phase I/2). Indeed, many of the promising complement drug candidates (e.g., compstatin and PMX-53) were initially developed in the academic field and further developed by biotechnology companies. Such partnerships have already been established in the case of ofatumumab (Genmab/Glaxo), pexelizumab (Alexion/Procter & Gamble, Cincinnati, OH, USA) and neutrazumab (G2 Therapies/Novo Nordisk).

ACKNOWLEDGMENTS

We thank Peter Ward, Wenchao Song and Maciej Markiewski for their input and for critically reading the manuscript and Deborah McClellan for editorial assistance. This work was supported by National Institutes of Health grants GM-069736, GM-62134, AI-30040, EB003968, CA112162 and AI-068730.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturebiotechnology/.

Published online at http://www.nature.com/naturebiotechnology/
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions

1. Nuttall, G. Experimente über die bacterienfeindlichen Einflüsse des thierischen Körpers. *Z. Hyg. Infektionskr.* **4**, 353–394 (1888).
2. Ehrlich, P. & Morgenroth, J. Ueber haemolysine---zweite mittheilung. *Berl. Klin. Wochenschr.*, 481–486 (1899).
3. US Food and Drug Administration. FDA approves first-of-its-kind drug to treat rare blood disorder (USFDA, Rockville, MD, USA) (http://www.fda.gov/bbs/topics/NEWS/2007/NEW01589.html) (March 16, 2007).
4. Sunyer, J.O., Zarkadis, I.K. & Lambris, J.D. Complement diversity: a mechanism for generating immune diversity? *Immunol. Today* **19**, 519–523 (1998).
5. Walport, M.J. Complement. First of two parts. *N. Engl. J. Med.* **344**, 1058–1066 (2001).
6. Walport, M.J. Complement. Second of two parts. *N. Engl. J. Med.* **344**, 1140–1144 (2001).
7. Longhi, M.P., Harris, C.L., Morgan, B.P. & Gallimore, A. Holding T cells in check–a new role for complement regulators? *Trends Immunol.* **27**, 102–108 (2006).
8. Morgan, B.P., Marchbank, K.J., Longhi, M.P., Harris, C.L. & Gallimore, A.M. Complement: central to innate immunity and bridging to adaptive responses. *Immunol. Lett.* **97**, 171–179 (2005).
9. Mastellos, D. & Lambris, J.D. Complement: more than a 'guard' against invading pathogens? *Trends Immunol.* **23**, 485–491 (2002).
10. Atkinson, J.P. & Frank, M.M. Bypassing complement: evolutionary lessons and future implications. *J. Clin. Invest.* **116**, 1215–1218 (2006).
11. Markiewski, M.M., Nilsson, B., Nilsson Ekdahl, K., Mollnes, T.E. & Lambris, J.D. Complement and coagulation: strangers or partners in crime? *Trends Immunol.* **28**, 184–192 (2007).
12. Hourcade, D.E. The role of properdin in the assembly of the alternative pathway C3 convertases of complement. *J. Biol. Chem.* **281**, 2128–2132 (2006).
13. Spitzer, D., Mitchell, L.M., Atkinson, J.P. & Hourcade, D.E. Properdin can initiate complement activation by binding specific target surfaces and providing a platform for de novo convertase assembly. *J. Immunol.* **179**, 2600–2608 (2007).
14. Kirkitadze, M.D. & Barlow, P.N. Structure and flexibility of the multiple domain proteins that regulate complement activation. *Immunol. Rev.* **180**, 146–161 (2001).
15. Soares, D.C. & Barlow, P.N. Complement control protein modules in the regulators of complement activation. in *Structural Biology of the Complement System*. (eds. Morikis, D. & Lambris, J.D.) 19–62 (CRC Press, Boca Raton, Florida, 2005).
16. Volanakis, J.E. & Frank, M. (eds.). *The Human Complement System in Health and Disease*. (Marcel Dekker, Inc., New York, 1998).
17. Sahu, A. & Lambris, J.D. Complement inhibitors: a resurgent concept in anti-inflammatory therapeutics. *Immunopharmacology* **49**, 133–148 (2000).
18. Markiewski, M.M. & Lambris, J.D. The role of complement in inflammatory diseases—from behind the scenes into the spotlight. *Am. J. Pathol.* **171**, 715–727 (2007).
19. Manderson, A.P., Botto, M. & Walport, M.J. The role of complement in the development of systemic lupus erythematosus. *Annu. Rev. Immunol.* **22**, 431–456 (2004).
20. Guo, R.F. & Ward, P.A. Role of C5a in inflammatory responses. *Annu. Rev. Immunol.* **23**, 821–852 (2005).

© 2007 Nature Publishing Group http://www.nature.com/naturebiotechnology

Samsung Bioepis Exhibit No. 1002
Page 994

© 2007 Nature Publishing Group http://www.nature.com/naturebiotechnology

21. Bonifati, D.M. & Kishore, U. Role of complement in neurodegeneration and neuroinflammation. *Mol. Immunol.* **44**, 999–1010 (2007).
22. Zipfel, P.F., Heinen, S., Jozsi, M. & Skerka, C. Complement and diseases: defective alternative pathway control results in kidney and eye diseases. *Mol. Immunol.* **43**, 97–106 (2006).
23. Davis, A.E., III. The pathophysiology of hereditary angioedema. *Clin. Immunol.* **114**, 3–9 (2005).
24. Wagenaar-Bos, I.G. & Hack, C.E. Structure and function of C1-inhibitor. *Immunol. Allergy Clin. North Am.* **26**, 615–632 (2006).
25. Hill, A., Richards, S.J. & Hillmen, P. Recent developments in the understanding and management of paroxysmal nocturnal haemoglobinuria. *Br. J. Haematol.* **137**, 181–192 (2007).
26. Rooijakkers, S.H. & van Strijp, J.A. Bacterial complement evasion. *Mol. Immunol.* **44**, 23–32 (2007).
27. Datta, P.K. & Rappaport, J. HIV and complement: hijacking an immune defense. *Biomed. Pharmacother.* **60**, 561–568 (2006).
28. Hammel, M. *et al.* A structural basis for complement inhibition by *Staphylococcus aureus*. *Nat. Immunol.* **8**, 430–437 (2007).
29. de Haas, C.J. *et al.* Chemotaxis inhibitory protein of Staphylococcus aureus, a bacterial antiinflammatory agent. *J. Exp. Med.* **199**, 687–695 (2004).
30. Rooijakkers, S.H. *et al.* Immune evasion by a staphylococcal complement inhibitor that acts on C3 convertases. *Nat. Immunol.* **6**, 920–927 (2005).
31. Bureeva, S., Andia-Pravdivy, J. & Kaplun, A. Drug design using the example of the complement system inhibitors' development. *Drug Discov. Today* **10**, 1535–1542 (2005).
32. Makrides, S.C. Therapeutic inhibition of the complement system. *Pharmacol. Rev.* **50**, 59–87 (1998).
33. Lambris, J.D. & Holers, V.M. (eds.) *Therapeutic Interventions in the Complement System* (Humana Press, Totowa, NJ, USA, 2000).
34. Holland, M.C., Morikis, D. & Lambris, J.D. Synthetic small-molecule complement inhibitors. *Curr. Opin. Investig. Drugs* **5**, 1164–1173 (2004).
35. Arkin, M.R. & Wells, J.A. Small-molecule inhibitors of protein-protein interactions: progressing towards the dream. *Nat. Rev. Drug Discov.* **3**, 301–317 (2004).
36. Sim, R.B. & Tsiftsoglou, S.A. Proteases of the complement system. *Biochem. Soc. Trans.* **32**, 21–27 (2004).
37. Narayana, S.V., Babu, Y.S. & Volanakis, J.E. Inhibition of complement serine proteases as a therapeutic strategy. in *Therapeutic Interventions in the Complement System*. (eds. J.D. Lambris & V.M. Holers) 57–74 (Humana Press, Totowa, New Jersey, 2000).
38. Szalai, A.J. *et al.* The Arthus reaction in rodents: species-specific requirement of complement. *J. Immunol.* **164**, 463–468 (2000).
39. Buerke, M., Schwertz, H., Seitz, W., Meyer, J. & Darius, H. Novel small molecule inhibitor of C1s exerts cardioprotective effects in ischemia-reperfusion injury in rabbits. *J. Immunol.* **167**, 5375–5380 (2001).
40. Agostoni, A., Cicardi, M., Bergamaschini, L., Boccassini, G. & Tucci, A. C1-inhibitor concentrate for treatment of hereditary angioedema. *N. Engl. J. Med.* **303**, 527 (1980).
41. Kirschfink, M. & Molines, T.E. C1-inhibitor: an anti-inflammatory reagent with therapeutic potential. *Expert Opin. Pharmacother.* **2**, 1073–1083 (2001).
42. De Serres, J., Groner, A. & Lindner, J. Safety and efficacy of pasteurized C1 inhibitor concentrate (Berinert P) in hereditary angioedema: a review. *Transfus. Apher. Sci.* **29**, 247–254 (2003).
43. Longhurst, H.J., Carr, S. & Khair, K. C1-inhibitor concentrate home therapy for hereditary angioedema: a viable, effective treatment option. *Clin. Exp. Immunol.* **147**, 11–17 (2007).
44. Bork, K., Barnstedt, S.E., Koch, P. & Traupe, H. Hereditary angioedema with normal C1-inhibitor activity in women. *Lancet* **356**, 213–217 (2000).
45. Pensky, J., Levy, L.R. & Lepow, I.H. Partial purification of a serum inhibitor of C'1-esterase. *J. Biol. Chem.* **236**, 1674–1679 (1961).
46. Caliezi, C. *et al.* C1-Esterase inhibitor: an anti-inflammatory agent and its potential use in the treatment of diseases other than hereditary angioedema. *Pharmacol. Rev.* **52**, 91–112 (2000).
47. Nielsen, E.W. *et al.* Effect of supraphysiologic levels of C1-inhibitor on the classical, lectin and alternative pathways of complement. *Mol. Immunol.* **44**, 1819–1826 (2007).
48. Lauterbach, M. *et al.* C1-esterase inhibitor reverses functional consequences of superior mesenteric artery ischemia/reperfusion by limiting reperfusion injury and restoring microcirculatory perfusion. *Shock* **27**, 75–83 (2007).
49. Lev Pharmaceuticals. Lev Pharmaceuticals reports positive results in pivotal phase III trial for hereditary angioedema (Lev Pharmaceuticals, New York) (http://www.levpharma.com/investors.news.3.14.07.aspx) (March 14, 2007).
50. Zuraw, B.L. Novel therapies for hereditary angioedema. *Immunol. Allergy Clin. North Am.* **26**, 691–708 (2006).
51. van Doorn, M.B. *et al.* A phase I study of recombinant human C1 inhibitor in asymptomatic patients with hereditary angioedema. *J. Allergy Clin. Immunol.* **116**, 876–883 (2005).
52. Brook, E., Herbert, A.P., Jenkins, H.T., Soares, D.C. & Barlow, P.N. Opportunities for new therapies based on the natural regulators of complement activation. *Ann. NY Acad. Sci.* **1056**, 176–188 (2005).
53. Weisman, H.F. *et al.* Soluble human complement receptor type 1: in vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. *Science* **249**, 146–151 (1990).
54. Li, J.S., Jaggers, J. & Anderson, P.A. The use of TP10, soluble complement receptor 1, in cardiopulmonary bypass. *Expert Rev. Cardiovasc. Ther.* **4**, 649–654 (2006).
55. Avant Immunotherapeutics, Inc. Press Release: AVANT Restructures Organization to Focus Resources on Core Programs and Operations (Avant Immunotherapeutics, Needham, MA) http://phx.corporate-ir.net/phoenix.zhtml?c=93243&p=irol-newsArticle&t=Regular&id=985098& (April 16, 2007).
56. Rittershaus, C.W. *et al.* Recombinant glycoproteins that inhibit complement activation and also bind the selectin adhesion molecules. *J. Biol. Chem.* **274**, 11237–11244 (1999).
57. Smith, R.A. Targeting anticomplement agents. *Biochem. Soc. Trans.* **30**, 1037–1041 (2002).
58. Hill, A. *et al.* Protection of erythrocytes from human complement-mediated lysis by membrane-targeted recombinant soluble CD59: a new approach to PNH therapy. *Blood* **107**, 2131–2137 (2006).
59. Presta, L.G. Selection, design, and engineering of therapeutic antibodies. *J. Allergy Clin. Immunol.* **116**, 731–736 (2005).
60. Frei, Y., Lambris, J.D. & Stockinger, B. Generation of a monoclonal antibody to mouse C5 application in an ELISA assay for detection of anti-C5 antibodies. *Mol. Cell. Probes* **1**, 141–149 (1987).
61. Wang, Y., Rollins, S.A., Madri, J.A. & Matis, L.A. Anti-C5 monoclonal antibody therapy prevents collagen-induced arthritis and ameliorates established disease. *Proc. Natl. Acad. Sci. USA* **92**, 8955–8959 (1995).
62. Alexion Pharmaceuticals, Inc., FDA approves Alexion's Soliris(TM) for all patients with PNH—first therapy approved for this rare and life-threatening blood disease (Alexion, Cheshire, CT) http://ir.alexionpharm.com/releasedetail.cfm?ReleaseID=234156 (March 16, 2007).
63. Thomas, T.C. *et al.* Inhibition of complement activity by humanized anti-C5 antibody and single-chain Fv. *Mol. Immunol.* **33**, 1389–1401 (1996).
64. Proctor, L.M., Woodruff, T.M. & Taylor, S.M. Recent developments in C5/C5a inhibitors. *Expert Opin. Ther. Pat.* **16**, 445–458 (2006).
65. Adis International Ltd. Eculizumab. *Drugs R D.* **8**, 61–68 (2007).
66. Whiss, P.A. Pexelizumab Alexion. *Curr. Opin. Investig. Drugs* **3**, 870–877 (2002).
67. Armstrong, P.W. *et al.* Pexelizumab for acute ST-elevation myocardial infarction in patients undergoing primary percutaneous coronary intervention: a randomized controlled trial. *J. Am. Med. Assoc.* **297**, 43–51 (2007).
68. Morrow, T. A promising theory stumbles in clinical trials. *Manag. Care* **16**, 69–70 (2007).
69. Steve Mitchell. Analysis: Alexion's pexelizumab fails (January 2, 2007) http://www.upi.com/Health_Business/Analysis/2007/01/02/analysis_alexions_pexelizumab_fails/6113/
70. Taube, C. *et al.* Factor B of the alternative complement pathway regulates development of airway hyperresponsiveness and inflammation. *Proc. Natl. Acad. Sci. USA* **103**, 8084–8089 (2006).
71. Teeling, J.L. *et al.* Characterization of new human CD20 monoclonal antibodies with potent cytolytic activity against non-Hodgkin lymphomas. *Blood* **104**, 1793–1800 (2004).
72. Teeling, J.L. *et al.* The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. *J. Immunol.* **177**, 362–371 (2006).
73. Sahu, A., Kay, B.K. & Lambris, J.D. Inhibition of human complement by a C3-binding peptide isolated from a phage-displayed random peptide library. *J. Immunol.* **157**, 884–891 (1996).
74. Janssen, B.J., Halff, E.F., Lambris, J.D. & Gros, P. Structure of compstatin in complex with complement component C3c reveals a new mechanism of complement inhibition. *J. Biol. Chem.* **282**, 29241–29247 (2007).
75. Sahu, A., Morikis, D. & Lambris, J.D. Compstatin, a peptide inhibitor of complement, exhibits species-specific binding to complement component C3. *Mol. Immunol.* **39**, 557–566 (2003).
76. Katragadda, M., Magotti, P., Sfyroera, G. & Lambris, J.D. Hydrophobic effect and hydrogen bonds account for the improved activity of a complement inhibitor, compstatin. *J. Med. Chem.* **49**, 4616–4622 (2006).
77. Potentia Pharmaceuticals, Inc., Potentia Pharmaceuticals announces initiation of phase I clinical trials to evaluate its lead compound for age-related macular degeneration (Potentia, Louisville, KY) http://www.prnewswire.com/cgi-bin/stories.pl?ACCT=104&STORY=/www/story/03-20-2007/0004549227&EDATE= (March 20, 2007).
78. Biesecker, G., Dihel, L., Enney, K. & Bendele, R.A. Derivation of RNA aptamer inhibitors of human complement C5. *Immunopharmacology* **42**, 219–230 (1999).
79. Bunka, D.H. & Stockley, P.G. Aptamers come of age—at last. *Nat. Rev. Microbiol.* **4**, 588–596 (2006).
80. Ng, E.W. *et al.* Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. *Nat. Rev. Drug Discov.* **5**, 123–132 (2006).
81. Monk, P.N., Scola, A.M., Madala, P. & Fairlie, D.P. Function, structure and therapeutic potential of complement C5a receptors. *Br. J. Pharmacol.*, **152**, 429–448 (2007).
82. Chen, N.J. *et al.* C5L2 is critical for the biological activities of the anaphylatoxins C5a and C3a. *Nature* **446**, 203–207 (2007).
83. Allegretti, M. *et al.* Targeting C5a: recent advances in drug discovery. *Curr. Med. Chem.* **12**, 217–236 (2005).
84. Wong, A.K., Taylor, S.M. & Fairlie, D.P. Development of C5a receptor antagonists. *IDrugs* **2**, 686–693 (1999).
85. Kohl, J. Drug evaluation: the C5a receptor antagonist PMX-53. *Curr. Opin. Mol. Ther.* **8**, 529–538 (2006).
86. March, D.R. *et al.* Potent cyclic antagonists of the complement C5a receptor on human polymorphonuclear leukocytes. Relationships between structures and activity. *Mol. Pharmacol.* **65**, 868–879 (2004).

Samsung Bioepis Exhibit No. 1002
Page 995

87. Woodruff, T.M. *et al.* Therapeutic activity of C5a receptor antagonists in a rat model of neurodegeneration. *FASEB J.* **20**, 1407–1417 (2006).
88. Petersen, K.A. *et al.* Phase I safety, tolerability, and pharmacokinetic study of recombinant human mannan-binding lectin. *J. Clin. Immunol.* **26**, 465–475 (2006).
89. Bureeva, S. *et al.* Selective inhibition of the interaction of C1q with immunoglobulins and the classical pathway of complement activation by steroids and triterpenoids sulfates. *Bioorg. Med. Chem.* **15**, 3489–3498 (2007).
90. Janssen, B.J. *et al.* Structures of complement component C3 provide insights into the function and evolution of immunity. *Nature* **437**, 505–511 (2005).
91. Janssen, B.J., Christodoulidou, A., McCarthy, A., Lambris, J.D. & Gros, P. Structure of C3b reveals conformational changes that underlie complement activity. *Nature* **444**, 213–216 (2006).
92. Wiesmann, C. *et al.* Structure of C3b in complex with CRIg gives insights into regulation of complement activation. *Nature* **444**, 217–220 (2006).
93. Nagar, B., Jones, R.G., Diefenbach, R.J., Isenman, D.E. & Rini, J.M. X-ray crystal structure of C3d: a C3 fragment and ligand for complement receptor 2. *Science* **280**, 1277–1281 (1998).
94. Milder, F.J. *et al.* Factor B structure provides insights into activation of the central protease of the complement system. *Nat. Struct. Mol. Biol.* **14**, 224–228 (2007).
95. Narayana, S.V. *et al.* Structure of human factor D. A complement system protein at 2.0 A resolution. *J. Mol. Biol.* **235**, 695–708 (1994).
96. Katschke, K.J. Jr. *et al.* A novel inhibitor of the alternative pathway of complement reverses inflammation and bone destruction in experimental arthritis. *J. Exp. Med.* **204**, 1319–1325 (2007).
97. Keller, T.H., Pichota, A. & Yin, Z. A practical view of 'druggability'. *Curr. Opin. Chem. Biol.* **10**, 357–361 (2006).
98. Lipinski, C.A., Lombardo, F., Dominy, B.W. & Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **46**, 3–26 (2001).
99. Hopkins, A.L. & Groom, C.R. The druggable genome. *Nat. Rev. Drug Discov.* **1**, 727–730 (2002).
100. Gould Rothberg, B.E., Pena, C.E.A. & Rothberg, J.M. A systems biology approach to target identification and validation for human chronic disease drug discovery. *Modern Biopharmaceuticals*, vol. 1 (ed. Knäblein, J.) 99–125 (Wiley-VCH Verlag, 2005).
101. Adams, C.P. & Brantner, V.V. Estimating the cost of new drug development: is it really 802 million dollars? *Health Aff. (Millwood)* **25**, 420–428 (2006).
102. Haffner, M.E., Whitley, J. & Moses, M. Two decades of orphan product development. *Nat. Rev. Drug Discov.* **1**, 821–825 (2002).
103. Title 21. United States Code (USC) Section 360ee.

© 2007 Nature Publishing Group http://www.nature.com/naturebiotechnology

Samsung Bioepis Exhibit No. 1002
Page 996

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 November 2005 (24.11.2005)

PCT

(10) International Publication Number
WO 2005/110481 A2

(51) International Patent Classification[7]: A61K 45/06

(21) International Application Number:
PCT/US2005/017048

(22) International Filing Date: 16 May 2005 (16.05.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/571,444 14 May 2004 (14.05.2004) US

(71) Applicants *(for all designated States except US)*: **ALEXION PHARMACEUTICALS, INC.** [US/US]; 352 Knotter Drive, Cheshire, CT 06410 (US). **THE UNIVERSITY OF WESTERN ONTARIO** [CA/CA]; 1151 Richmond Street North, Stevenson-Lawson Building, Room 328, London, Ontario N6A 5B8 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ROTHER, Russell, P.** [US/US]; 25 Blue Trail Drive, Prospect, CT 06712 (US). **WANG, Hao** [CA/CA]; 480 Sandybrook Drive, London, Ontario N5X 4H2 (CA). **ZHONG, Zhen** [CA/CA]; 60 Nathaniel Court, London, Ontario N5X 2N5 (CA).

(74) Agents: **WU, Xuqiong** et al.; Fish & Neave IP Group of, Ropes & Gray LLP, One International Place, Boston, MA 02110-2624 (US).

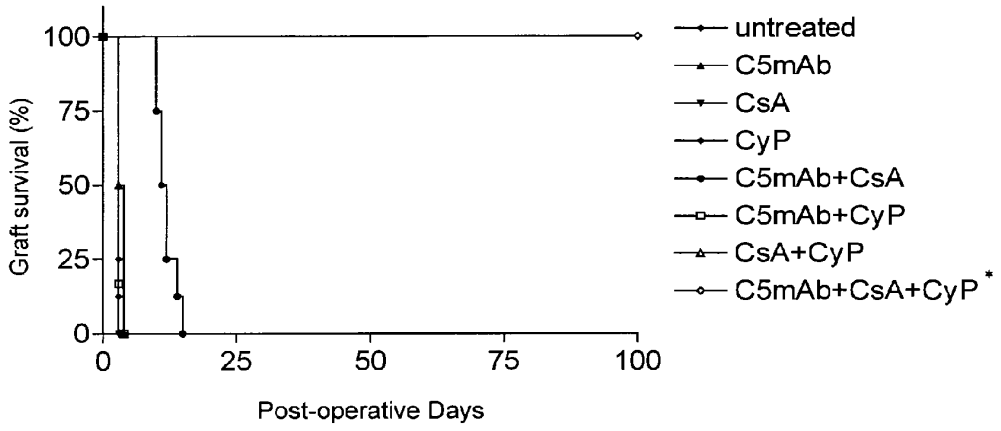
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PROLONGATION OF SURVIVAL OF AN ALLOGRAFT BY INHIBITING COMPLEMENT ACTIVITY



(57) Abstract: Methods of prolonging survival of allotransplanted cells, tissues or organs are presented. These methods are directed to administering to the allotransplant recipient an inhibitor of complement activity together with one or more immunosuppressants. The inhibitor of complement activity is administered chronically. These methods have been determined to aid in preventing chronic rejection of allografts. These methods can additionally be used in cases in which the recipient has been presensitized to the allograft or in which there is an ABO mismatch between the allograft and the recipient.

WO 2005/110481 A2

PROLONGATION OF SURVIVAL OF AN ALLOGRAFT BY INHIBITING COMPLEMENT ACTIVITY

RELATED APPLICATIONS

This application claims the benefit of and priority to U.S. Provisional Patent Application No. 60/571,444, filed May 14, 2004, the disclosure of which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

The present disclosure relates to methods for prolonging survival of an allograft in a mammal. In particular, the present disclosure relates to prolonging survival of an allograft by administering an inhibitor of complement or terminal complement formation, especially an inhibitor of complement C5 cleavage, in addition to one or more drugs that are immunosuppressant.

BACKGROUND

Organ transplantation is the preferred treatment for most patients with chronic organ failure. Although transplantation of kidney, liver, lung, and heart offers an excellent opportunity for rehabilitation as recipients return to a more normal lifestyle, it is limited by the medical/surgical suitability of potential recipients, an increasing shortage of donors, and premature failure of transplanted organ function.

Transplantation of cells, tissues and organs has become very common and is often a life-saving procedure. Organ transplantation is the preferred treatment for most patients with chronic organ failure. Despite great improvement in treatments to inhibit rejection, rejection continues to be the single largest impediment to successful organ transplantation. Rejection includes not only acute rejection but also chronic rejection. One-year survival rates for transplanted kidneys average 88.3% with kidneys from deceased donors and 94.4% with kidneys received from living donors. The corresponding five year survival rates for the transplanted kidneys are 63.3% and 76.5% (OPTN/SRTR Annual Report, 2002). For livers the one year survival rates are 80.2% and 76.5% for livers from deceased and living donors, respectively. The corresponding five year liver graft survival rates are 63.5% and 73.0% (OPTN/SRTR Annual Report, 2002). The use of immunosuppressant drugs, especially cyclosporin A and more recently tacrolimus, has dramatically improved the success rate of organ transplantation especially by preventing acute rejection. But as the numbers above

Samsung Bioepis Exhibit No. 1002
Page 998

show, there is still a need to improve the success rates, both short-term and especially long-term. As seen from the above numbers for kidney and liver transplants, the five year failure rates for these transplanted organs are on the order of 25-35%. In the year 2001 alone there were more than 23,000 patients who received an organ transplant of which approximately 19,000 were kidney or liver (OPTN/SRTR Annual Report, 2002). For this one year of transplants alone, with present techniques it can be expected that approximately 5,000-6,000 of these transplanted kidneys and livers will fail within 5 years. These numbers do not even include other transplanted organs or transplanted tissues or cells such as bone marrow.

There are multiple types of transplants. These are described in Abbas et al., 2000. A graft transplanted from one individual to the same individual is called an autologous graft or autograft. A graft transplanted between two genetically identical or syngeneic individual is called a syngeneic graft. A graft transplanted between two genetically different individuals of the same species is called an allogeneic graft or allograft. A graft transplanted between individuals of different species is called a xenogeneic graft or xenograft. The molecules that are recognized as foreign on allografts are called alloantigens and those on xenografts are called xenoantigens. The lymphocytes or antibodies that react with alloantigens or xenoantigens are described as being alloreactive or xenoreactive, respectively.

Currently more than 40,000 kidney, heart, lung, liver and pancreas transplants are performed in the United States each year (Abbas et al., 2000). Other possible transplants include, but are not limited to, vascular tissue, eye, cornea, lens, skin, bone marrow, muscle, connective tissue, gastrointestinal tissue, nervous tissue, bone, stem cells, islets, cartilage, hepatocytes, and hematopoietic cells. Unfortunately, there are many more candidates for a transplant than there are donors. To overcome this shortage, a major effort is being made to learn how to use xenografts. While progress is being made in this field, the fact is that at present most transplants are allografts. An allogeneic transplant, while presently being more likely to be successful than a xenogeneic transplant, must surmount numerous obstacles to be successful. There are several types of immunological attacks made by the recipient against the donor organ which can lead to rejection of the allograft. These include hyperacute rejection, acute vascular rejection (including accelerated humoral rejection and de novo acute humoral rejection), and chronic rejection. Rejection is normally a result of T-cell mediated or humoral antibody attack, but may include additional secondary factors such as the effects of complement and cytokines.

Samsung Bioepis Exhibit No. 1002
Page 999

An ever growing gap between the number of patients requiring organ transplantation and the number of donor organs available has become a major problem throughout the world. Park et al., 2003. Individuals who have developed anti-HLA antibodies are said to be immunized or sensitized. Gloor, 2005. HLA sensitization is the major barrier to optimal utilization of organs from living donors in clinical transplantation (Warren et al., 2004) due to the development of severe antibody-mediated rejection (ABMR). For example, more than 50% of all individuals awaiting kidney transplantation are presensitized patients (Glotz et al., 2002) who have elevated levels of broadly reactive alloantibodies, resulting from multiple transfusions, prior failed allografts, or pregnancy (Kupiec-Weglinski, 1996). The role of ABMR is currently one of the most dynamic areas of study in transplantation, due to recognition that this type of rejection can lead to either acute or chronic loss of allograft function. Mehra et al., 2003. Numerous cases of ABMR, including hyperacute rejection (HAR) or accelerated humoral rejection (ACHR), have been reported that are characterized by acute allograft injury that is resistant to potent anti-T cell therapy, the detection of circulating donor specific antibodies, and the deposition of complement components in the graft. ABMR with elevated circulating alloantibodies and complement activation that occurs in 20-30% of acute rejection cases has a poorer prognosis than cellular rejection. Mauiyyedi et al., 2002.

Highly presensitized patients, who exhibit high levels of alloantibodies, usually suffer an immediate and aggressive HAR. In clinical practice, with great efforts and significant advances in technology, HAR is avoided by obtaining a pretransplant lymphocytotoxic cross-match to identify sensitized patients with antibodies specific for donor HLA antigens. However, circulating antibodies against donor HLA or other non-MHC endothelial antigens may also be responsible for a delayed form of acute humoral rejection, which is associated with an increased incidence of graft loss. Collins et al., 1999. Therefore, development of a novel presensitized animal model to mimic ABMR in clinical settings would be beneficial to studies on the mechanism, and to the much needed progress in the management of allograft rejection in presensitized hosts.

Some highly presensitized patients can benefit from intervention programs such as immunoadsorption (Palmer et al.,1989; Ross et al., 1993; Kriaa et al., 1995), plasmapheresis and intravenous immunoglobulin (Sonnenday et al., 2002; Rocha et al., 2003), that have been designed and implemented to temporarily eliminate anti-donor antibodies. However, in addition to their benefits, the aforementioned therapies carry with them numerous drawbacks

Samsung Bioepis Exhibit No. 1002
Page 1000

as some individuals are less susceptible to their effects (Kriaa et al., 1995; Hakim et al., 1990; Glotz et al., 1993; Tyan et al., 1994); they are extremely expensive, time-consuming, and risky (Salama et al., 2001). Moreover, the transient and variable effect of these protocols has limited their impact. Glotz et al., 2002; Kupin et al., 1991; Schweitzer et al., 2000. Therefore, developing novel strategies to reduce the risk and cost in prevention of ABMR would be beneficial to presensitized recipients receiving an allograft.

SUMMARY

Accordingly, methods of prolonging survival of transplanted cells, tissues or organs are provided. In particular, methods of prolonging survival of allotransplanted cells, tissues or organs are provided. These methods are directed to using one or more immunosuppressants in addition to an inhibitor of complement activity. Use of one or more immunosuppressants and an inhibitor of complement activity in the manufacture of one or more medicaments or medicament packages is also provided. Such medicaments or medicament packages are useful in prolonging survival of an allograft in a subject mammal.

In certain embodiments, the inhibition of complement activity is effected by chronic administration of a drug directed against complement C5. A preferred drug that inhibits complement activity is an antibody specific to one or more components of complement, for example, C5. In certain preferred embodiments, the antibody inhibits the cleavage of C5 and thereby inhibits the formation of both C5a and C5b-9. The antibody may be, e.g., a monoclonal antibody, a chimeric antibody (e.g., a humanized antibody), an antibody fragment (e.g., Fab), a single chain antibody, an Fv, or a domain antibody. The recipient is also treated with one or more immunosuppressive drugs, for example, cyclosporin A.

In certain embodiments, either an MHC mismatched recipient (i.e., a mammalian recipient of an MHC mismatched allograft), a presensitized recipient or an ABO mismatched recipient (i.e., a mammalian recipient of an AMB mismatched allograft) is treated. In this model, the recipient is again chronically treated with a complement inhibitor, preferably an anti-C5 monoclonal antibody, together with immunosuppressive drugs, preferably a chronic administration of cyclosporin A and a short-term administration of cyclophosphamide. This triple therapy results in extended graft survival in the presensitized allotransplant recipient.

The present disclosure also provides methods of prolonging survival of an allograft in a mammalian recipient by administering to the recipient agents that modulate the level and/or ratio of subclasses and/or isotypes of anti-donor immunoglobulins (Ig) in the recipient. In

Samsung Bioepis Exhibit No. 1002
Page 1001

certain embodiments, an agent that reduces the level of anti-donor IgG1 in the recipient is preferred. In certain embodiments, an agent that increases the level of anti-donor IgG2a and/or IgG2b in the recipient is preferred. In certain embodiments, an agent that reduces the ratio of anti-donor IgG1/anti-donor IgG2a or IgG2b in the recipient is preferred.

The present disclosure also provides a method of prolonging survival of an allograft in a second mammalian recipient using an allograft that has been accommodated in a first mammalian recipient (i.e., the allograft has prolonged survival in the first recipient). The present disclosure further provides an allograft that is resistant to anti-donor antibodies in a mammalian recipient, and the allograft is prepared from a first recipient that has accommodated the allograft. In preferred embodiments, the first recipient has accommodated the allograft by receiving a treatment as described herein, such a triple therapy treatment involving administering to the first recipient a drug that inhibits complement activity and two immunosuppressive agents.

Further provided are pharmaceutical packages. A pharmaceutical package of the present disclosure may comprise a drug that inhibits complement activity and at least one immunosuppressive agent. The pharmaceutical package may further comprise a label for chronic administration. The pharmaceutical package may also comprise a label for self-administration by a patient, for example, a recipient of a transplant graft, or instructions for a caretaker of a recipient of a transplant graft. In certain embodiments, the drug and the agent in the pharmaceutical package are in a formulation or separate formulations that are suitable for chronic administration and/or self-administration.

The present disclosure also provides lyophilized formulations and formulations suitable for injection. Certain embodiments provide a lyophilized antibody formulation comprising an antibody that inhibits complement activity and a lyoprotectant. In preferred embodiments, the antibody formulation is suitable for chronic administration, for example, the antibody formulation is stable. Alternative embodiments provide an injection system comprising a syringe; the syringe comprises a cartridge containing an antibody that inhibits complement activity and is in a formulation suitable for injection.

An antibody employed in various embodiments of the present disclosure preferably inhibits the formation of terminal complement or C5a. In certain embodiments, antibody inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment. The whole antibody or antibody fragment may be a human, humanized,

Samsung Bioepis Exhibit No. 1002
Page 1002

chimerized or deimmunized antibody or antibody fragment. In certain embodiments, the whole antibody or antibody fragment may inhibit cleavage of complement C5. In certain embodiments, the antibody fragment is a Fab, an F(ab')2, an Fv, a domain antibody, or a single-chain antibody. In preferred embodiments, the antibody fragment is pexelizumab. In alternative preferred embodiments, the whole antibody is eculizumab.

In certain embodiments, a drug, such as an antibody, that inhibits complement activity is present in unit dosage form, which can be particularly suitable for self-administration. Similarly, an immunosuppressive agent of the present disclosure may also be present in unit dosage form.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D show anti-donor antibody levels in presensitized versus unsensitized recipients under different treatments.

Figures 2A and 2B show comparison between triple therapy using anti-C5 antibody, CsA and CyP in presensitized allograft recipients and combination therapy using only anti-C5 antibody and CsA in presensitized allograft recipients. Figure 2A compares heart-allograft survival in various recipients under different treatments as indicated. Figure 2B shows histology and immunohistology, for example, for lymphocyte infiltration in heart allografts of recipients in different groups.

Figure 3 shows blocked terminal complement activity by anti-C5 antibody as compared to immunosuppressive agents.

Figures 4A-4D compare levels of anti-donor antibodies in presensitized recipients of allografts under monotherapy with anti-C5 antibody alone, double combination therapy with anti-C5 antibody and CsA, and triple combination therapy with anti-C5 antibody, CsA and CyP.

Figures 5A and 5B show change of ratios of IgG isotypes in allograft recipients that were untreated or under different treatments.

Figure 6 shows high-level expression of Bcl-2 and Bcl-xl proteins in long-term surviving heart grafts as compared to heart grafts of untreated animals.

Figure 7 shows improved second transplantation (re-transplantation) of an accommodated graft from a first transplantation recipient.

Figure 8 shows results from re-transplantation experiments.

Samsung Bioepis Exhibit No. 1002
Page 1003

Figure 9 shows results from re-transplantation experiments.

DETAILED DESCRIPTION

Overview: Rejection of Transplants or Grafts

Hyperacute rejection occurs within minutes to hours after transplant and is due to preformed antibodies to the transplanted tissue antigens. It is characterized by hemorrhage and thrombotic occlusion of the graft vasculature. The binding of antibody to endothelium activates complement, and antibody and complement induce a number of changes in the graft endothelium that promote intravascular thrombosis and lead to vascular occlusion, the result being that the grafted organ suffers irreversible ischemic damage (Abbas et al., 2000). Hyperacute rejection is often mediated by preexisting IgM alloantibodies, e.g., those directed against the ABO blood group antigens expressed on red blood cells. This type of rejection, mediated by natural antibodies, is the main reason for rejection of xenotransplants. Hyperacute rejection due to natural IgM antibodies is no longer a major problem with allografts because allografts are usually selected to match the donor and recipient ABO type. Hyperacute rejection of an ABO matched allograft may still occur, usually mediated by IgG antibodies directed against protein alloantigens, such as foreign MHC molecules, or against less well defined alloantigens expressed on vascular endothelial cells. Such antibodies may arise as a result of prior exposure to alloantigens through blood transfusion, prior transplantation, or multiple pregnancies (this prior exposure being referred to as "presensitization"). Abbas et al., 2000.

Acute rejection is a process of vascular and parenchymal injury mediated by T cells, macrophages, and antibodies that usually begins after the first week of transplantation. Abbas et al., 2001. T lymphocytes play a central role in acute rejection by responding to alloantigens, including MHC molecules, present on vascular endothelial and parenchymal cells. The activated T cells cause direct lysis of graft cells or produce cytokines that recruit and activate inflammatory cells, which cause necrosis. Both $CD4^+$ and $CD8^+$ cells may contribute to acute rejection. The destruction of allogeneic cells in a graft is highly specific and a hallmark of $CD8^+$ cytotoxic T lymphocyte killing. Abbas et al., 2000. $CD4^+$ T cells may be important in mediating acute graft rejection by secreting cytokines and inducing delayed-type hypersensitivity-like reactions in grafts, with some evidence available that indicates that $CD4^+$ T cells are sufficient to mediate acute rejection. Abbas et al., 2000. Antibodies can also mediate acute rejection after a graft recipient mounts a humoral immune

Samsung Bioepis Exhibit No. 1002
Page 1004

response to vessel wall antigens and the antibodies that are produced bind to the vessel wall and activate complement. Abbas et al., 2000.

Chronic rejection is characterized by fibrosis with loss of normal organ structures occurring over a prolonged period. The pathogenesis of chronic rejection is less well understood than that of acute rejection. Graft arterial occlusion may occur as a result of the proliferation of intimal smooth muscle cells (Abbas et al., 2000). This process is called accelerated or graft arteriosclerosis and can develop in any vascularized organ transplant within 6 months to a year after transplantation.

For a transplant to be successful, the several modes of rejection must be overcome. Multiple approaches are utilized in preventing rejection. This may require administration of immunosuppressants, often several types to prevent the various modes of attack, e.g., inhibition of T-cell attack, antibodies, and cytokine and complement effects. Prescreening of donors to match them with recipients is also a major factor in preventing rejection, especially in preventing hyperacute rejection. Immunoadsorption of anti-HLA antibodies prior to grafting may reduce hyperacute rejection. Prior to transplantation the recipient or host may be administered anti-T cell reagents, e.g., the monoclonal antibody OKT3, Anti-Thymocyte Globulin (ATG), cyclosporin A, or tacrolimus (FK 506). Additionally, glucocorticoids and/or azathioprine may be administered to the host prior to transplant. Drugs used to aid in preventing transplant rejection include, but are not limited to, ATG or ALG, OKT3, daclizumab, basiliximab, corticosteroids, 15-deoxyspergualin, cyclosporins, tacrolimus, azathioprine, methotrexate, mycophenolate mofetil, 6-mercaptopurine, bredinin, brequinar, leflunamide, cyclophosphamide, sirolimus, anti-CD4 monoclonal antibodies, CTLA4-Ig, anti-CD154 monoclonal antibodies, anti-LFA1 monoclonal antibodies, anti-LFA-3 monoclonal antibodies, anti-CD2 monoclonal antibodies, and anti-CD45.

Allografts are rejected in part by the activation of T cells. The transplant recipient mounts a rejection response following $CD4^+$ T cell recognition of foreign antigens in the allograft. These antigens are encoded by the major histocompatibility complex (MHC). There are both Class I and Class II MHC molecules. In humans the class I MHC molecules are HLA-A, HLA-B, and HLA-C. The class II MHC molecules in humans are called HLA-DR, HLA-DQ and HLA-DP. In mice the class I MHC molecules are H-2K, H-2D and H-2L and the class II MHC molecules are I-A and I-E. When $CD4^+$ T cells bind the foreign MHC antigens they are activated and undergo clonal proliferation. The activated T cells secrete cytokines which aid in activating monocytes/macrophages, B cells and cytotoxic $CD8^+$ T

Samsung Bioepis Exhibit No. 1002
Page 1005

cells. The activated monocytes/macrophages release agents which result in tissue damage, the B cells produce alloantibodies which lead to complement mediated tissue destruction, and the $CD8^+$ T cells kill graft cells in an antigen-specific manner through induction of apoptosis and cell lysis.

Immunosuppressive Agents

The numerous drugs utilized to delay graft rejection (i.e., to prolong their survival) work in a variety of ways. Immunosuppressive agents are widely used. See Stepkowski, 2000, for a review of the mechanism of action of several immunosuppressive drugs. Cyclosporin A is one of the most widely used immunosuppressive drugs for inhibiting graft rejection. It is an inhibitor of interleukin-2 or IL-2 (it prevents mRNA transcription of interleukin-2). More directly, cyclosporin inhibits calcineurin activation that normally occurs upon T cell receptor stimulation. Calcineurin dephosphorylates NFAT (nuclear factor of activated T cells) enabling it to enter the nucleus and bind to interleukin-2 promoter. By blocking this process, cyclosporin A inhibits the activation of the $CD4^+$ T cells and the resulting cascade of events which would otherwise occur. Tacrolimus is another immunosuppressant that acts by inhibiting the production of interleukin-2.

Rapamycin (Sirolimus), SDZ RAD, and interleukin-2 receptor blockers are drugs that inhibit the action of interleukin-2 and therefore prevent the cascade of events described above.

Inhibitors of purine or pyrimidine biosynthesis are also used to inhibit graft rejection. These prevent DNA synthesis and thereby inhibit cell division including the ability of T cells to divide. The result is the inhibition of T cell activity by preventing the formation of new T cells. Inhibitors of purine synthesis include azathioprine, methotrexate, mycophenolate mofetil (MMF) and mizoribine (bredinin). Inhibitors of pyrimidine synthesis include brequinar sodium and leflunomide. Cyclophosphamide is an inhibitor of both purine and pyrimidine synthesis.

Yet another method for inhibiting T cell activation is to treat the recipient with antibodies to T cells. OKT3 is a murine monoclonal antibody against CD3 which is part of the T cell receptor. This antibody inhibits the T cell receptor and suppresses T cell activation.

Numerous other drugs and methods for delaying allotransplant rejection are known to and used by those of skill in the art. One approach has been to deplete T cells, e.g., by irradiation. This has often been used in bone marrow transplants, especially if there is a

Samsung Bioepis Exhibit No. 1002
Page 1006

partial mismatch of major HLA. Administration to the recipient of an inhibitor (blocker) of the CD40 ligand-CD40 interaction and/or a blocker of the CD28-B7 interaction has been used (U.S. Patent 6,280,957). Published PCT patent application WO 01/37860 teaches the administration of an anti-CD3 monoclonal antibody and IL-5 to inhibit the Th1 immune response. Published PCT patent application WO 00/27421 teaches a method for prophylaxis or treatment of corneal transplant rejection by administering a tumor necrosis factor-α antagonist. Glotz et al. (2002) show that administration of intravenous immunoglobulins (IVIg) can induce a profound and sustained decrease in the titers of anti-HLA antibodies thereby allowing a transplant of an HLA-mismatched organ. Similar protocols have included plasma exchanges (Taube et al., 1984) or immunoadsorption techniques coupled to immunosuppressive agents (Hiesse et al., 1992) or a combination of these (Montgomery et al., 2000). Changelian et al. (2003) teach a model in which immunosuppression is caused by an oral inhibitor of Janus kinase 3 (JAK3) which is an enzyme necessary for the proper signaling of cytokine receptors which use the common gamma chain (γc) (Interleukins-2, -4, -7, -9, -15, -21), the result being an inhibition of T cell activation. Antisense nucleic acids against ICAM-1 have been used alone or in combination with a monoclonal antibody specific for leukocyte-function associated antigen 1 (LFA-1) in a study of heart allograft transplantation (Stepkowski, 2000). Similarly, an anti-ICAM-1 antibody has been used in combination with anti-LFA-1 antibody to treat heart allografts (Stepkowski, 2000). Antisense oligonucleotides have additionally been used in conjunction with cyclosporin in rat heart or kidney allograft models, resulting in a synergistic effect to prolong the survival of the grafts (Stepkowski, 2000). Chronic transplant rejection has been treated by administering an antagonist of TGF-β which is a cytokine involved in differentiation, proliferation and apoptosis (U.S. Patent Application Publication US 2003/0180301).

Complement and Transplant/Graft Rejection

The role of complement in transplant rejection is well known. This is especially true in the case of xenotransplantation, but complement also plays a role in allotransplant rejection. For review, see Platt and Saadi, 1999. One aspect of complement's role is that ischemia-reperfusion injury may occur at the time that an organ graft is reperfused with the blood of the recipient. Complement may also cause some manifestations of allograft rejection.

The complement system is described in detail in U.S. Patent 6,355,245. The complement system acts in conjunction with other immunological systems of the body to

Samsung Bioepis Exhibit No. 1002
Page 1007

defend against intrusion of cellular and viral pathogens. There are at least 25 complement proteins, which are found as a complex collection of plasma proteins and membrane cofactors. The plasma proteins make up about 10% of the globulins in vertebrate serum. Complement components achieve their immune defensive functions by interacting in a series of intricate but precise enzymatic cleavage and membrane binding events. The resulting complement cascade leads to the production of products with opsonic, immunoregulatory, and lytic functions.

The complement cascade progresses via the classical pathway or the alternative pathway. These pathways share many components and, while they differ in their initial steps, they converge and share the same "terminal complement" components (C5 through C9) responsible for the activation and destruction of target cells.

The classical complement pathway is typically initiated by antibody recognition of and binding to an antigenic site on a target cell. The alternative pathway is usually antibody independent and can be initiated by certain molecules on pathogen surfaces. Both pathways converge at the point where complement component C3 is cleaved by an active protease (which is different in each pathway) to yield C3a and C3b. Other pathways activating complement attack can act later in the sequence of events leading to various aspects of complement function.

C3a is an anaphylatoxin. C3b binds to bacterial and other cells, as well as to certain viruses and immune complexes, and tags them for removal from the circulation. C3b in this role is known as opsonin. The opsonic function of C3b is considered to be the most important anti-infective action of the complement system. Patients with genetic lesions that block C3b function are prone to infection by a broad variety of pathogenic organisms, while patients with lesions later in the complement cascade sequence, i.e., patients with lesions that block C5 functions, are found to be more prone only to Neisseria infection, and then only somewhat more prone (Fearon, 1983).

C3b also forms a complex with other components unique to each pathway to form classical or alternative C5 convertase, which cleaves C5 into C5a and C5b. C3 is thus regarded as the central protein in the complement reaction sequence since it is essential to both the alternative and classical pathways (Wurzner et al., 1991). This property of C3b is regulated by the serum protease Factor I, which acts on C3b to produce iC3b. While still functional as opsonin, iC3b cannot form an active C5 convertase.

Samsung Bioepis Exhibit No. 1002
Page 1008

C5 is a 190 kDa beta globulin found in normal serum at approximately 75 μg/mL (0.4 μM). C5 is glycosylated, with about 1.5-3 percent of its mass attributed to carbohydrate. Mature C5 is a heterodimer of a 999 amino acid 115 kDa alpha chain that is disulfide linked to a 656 amino acid 75 kDa beta chain. C5 is synthesized as a single chain precursor protein product of a single copy gene (Haviland et al., 1991). The cDNA sequence of the transcript of this gene predicts a secreted pro-C5 precursor of 1659 amino acids along with an 18 amino acid leader sequence.

The pro-C5 precursor is cleaved after amino acid 655 and 659, to yield the beta chain as an amino terminal fragment (amino acid residues +1 to 655) and the alpha chain as a carboxyl terminal fragment (amino acid residues 660 to 1658), with four amino acids deleted between the two.

C5a is cleaved from the alpha chain of C5 by either alternative or classical C5 convertase as an amino terminal fragment comprising the first 74 amino acids of the alpha chain (i.e., amino acid residues 660-733). Approximately 20 percent of the 11 kDa mass of C5a is attributed to carbohydrate. The cleavage site for convertase action is at or immediately adjacent to amino acid residue 733. A compound that would bind at or adjacent to this cleavage site would have the potential to block access of the C5 convertase enzymes to the cleavage site and thereby act as a complement inhibitor.

C5 can also be activated by means other than C5 convertase activity. Limited trypsin digestion (Minta and Man, 1977; Wetsel and Kolb, 1982) and acid treatment (Yamamoto and Gewurz, 1978; Vogt et al., 1989) can also cleave C5 and produce active C5b.

C5a is another anaphylatoxin. C5b combines with C6, C7, and C8 to form the C5b-8 complex at the surface of the target cell. Upon binding of several C9 molecules, the membrane attack complex (MAC, C5b-9, terminal complement complex-TCC) is formed. When sufficient numbers of MACs insert into target cell membranes the openings they create (MAC pores) mediate rapid osmotic lysis of the target cells. Lower, non-lytic concentrations of MACs can produce other effects. In particular, membrane insertion of small numbers of the C5b-9 complexes into endothelial cells and platelets can cause deleterious cell activation. In some cases activation may precede cell lysis.

As mentioned above, C3a and C5a are anaphylatoxins. These activated complement components can trigger mast cell degranulation, which releases histamine and other mediators of inflammation, resulting in smooth muscle contraction, increased vascular

Samsung Bioepis Exhibit No. 1002
Page 1009

permeability, leukocyte activation, and other inflammatory phenomena including cellular proliferation resulting in hypercellularity. C5a also functions as a chemotactic peptide that serves to attract pro-inflammatory granulocytes to the site of complement activation.

Complement-binding recipient antibodies to donor alloantigens are considered to be the main cause of hyperacute graft rejection. Owing to pretransplant crossmatch testing, this prototype of humoral rejection is now rarely observed (Regele et al., 2001). Data are now showing that humoral immune mechanisms might contribute to other types of allograft rejection (Regele et al., 2001). High levels of panel reactive antibodies indicating humoral presensitization were found to be associated with inferior kidney graft survival (Opelz, 1992), the appearance of alloantibodies during the post-transplant period has been reported to predict poor graft outcome (Jeannet et al., 1970; Halloran et al., 1992), and selective removal of recipient IgG by immunoadsorption reversed some rejection episodes indicating the contribution of humoral immune mechanisms to rejection (Persson et al., 1995; Böhmig et al., 2000). Complement activation within a graft might indicate antibody-mediated graft injury. The complement cleavage product C4d is a marker for activation of the antibody-dependent classical pathway. Capillary C4d deposits in kidney allograft biopsies were associated with poor graft outcome.

Recently increasing evidence indicates that complement activation significantly contributes to the sensitization of allograft recipients and the development of tissue injury in allografts (Platt et al., 1999). Antibodies are the most thoroughly investigated mediators of activating the classical complement pathway. Clinically, alloantibodies are known to activate complement (Baldwin et al., 2001). Halloran and Collins indicate that C4d deposition in peritubular capillaries of renal allografts is a sensitive and diagnostic marker of acute humoral rejection that correlates strongly with the presence of circulating donor-specific antibodies (Collins et al., 1999; Halloran, 2003). Further supporting evidence is seen in animals with complement inhibition (Pratt et al., 1996; Pruitt et al., 1991; Forbes et al., 1978) or deficiency (Pratt et al., 2000; Baurer et al., 1995) which exhibit significantly reduced inflammatory injury and lowered anti-donor immune responses. In ABMR, complement is suggested to be activated by the classical pathway and to play a key role in the pathogenesis (Collard et al., 1997). Although the role of complement in HAR or acute vascular rejection (AVR) following xenotransplantation has been well documented (Platt et al., 1999), precise mechanisms of complement in the pathogenesis of ABMR following allotransplantation has not yet been elucidated.

Samsung Bioepis Exhibit No. 1002
Page 1010

The C5 component of complement is cleaved to form products with multiple proinflammatory effects and thus represents an attractive target for complement inhibition within the immune-mediated inflammatory response. As described above, C5a is a powerful anaphylatoxin and chemotactic factor. Cellular activation by C5a induces the release of multiple additional inflammatory mediators (Jose et al., 1983). The complement activation pathways (classical, alternative, or mannan-binding lectin pathway) ultimately lead to the formation of the cytolytic membrane attack complex C5b-9 (Kirschfunk, 2001), which can mediate both direct tissue injury by cell lysis, and proinflammatory cell activation at sublytic doses (Saadi et al., 1995; Papadimitriou et al., 1991). Therefore, blocking both C5a and C5b-9 generation may be required for the optimal inhibition of complement-mediated inflammatory response following transplantation. At the same time, inhibition of the complement cascade at C5 does not impair the generation of C3b, preserving C3b-mediated opsonization of pathogenic microorganisms as well as solubilization and clearance of immune complexes (Liszewski, 1993).

The beneficial effect of anti-C5 mAb has previously been reported in several experimental models including myocardial reperfusion (Vakeva et al., 1998), systemic lupus erythematosus (Wang et al., 1996) and rheumatoid arthritis (Wang et al., 1995); as well as in human clinical trials (Kirschfink, 2001) of autoimmune disease, cardiopulmonary bypass and acute myocardial infarction. In addition, complement inactivation by a functionally blocking anti-C5 monoclonal Ab (mAb) prevented HAR in xenotransplantation models (Kroshus et al., 1995; Wang et al., 1999).

Methods of delaying allotransplant rejection by administration of complement inhibitors have been tested. Published PCT patent application WO 92/10205 discloses the use of a combination of cyclosporin and a soluble complement receptor (sCR1) to inhibit rejection of a cardiac allotransplant in a presensitized rat model. Complement receptor 1 binds complements C3b and C4b. Soluble forms of complement receptor 1 occur naturally or can be generated via recombinant DNA procedures. These soluble complement receptors have inhibited in vitro the consequences of complement activation (U.S. Patent 6,057,131). In WO 92/10205, rats, which had been presensitized to the cardiac allograft they were receiving, were administered cyclosporin A intramuscularly at 10 mg/kg/day beginning two days prior to transplant and continued until the time of graft rejection. Additionally, soluble complement receptor 1 (sCR1) was administered as a single intravenous bolus at 15 mg/kg immediately prior to reperfusion of the graft. Control animals with no drug treatment had the

Samsung Bioepis Exhibit No. 1002
Page 1011

graft rejected at an average of 3.8 days. Those administered cyclosporin A alone rejected the grafts at an average of 57 days (this was quite variable with two rats rejecting quickly at 2 and 4 days and a third rat rejecting at 166 days). Rats administered sCR1 alone rejected the grafts at an average of 44 days. Those rats administered the combination of cyclosporin A and sCR1 rejected the grafts at an average of 147 days. The combination of chronic cyclosporin A and single bolus sCR1 was seen to result in a synergistic effect greatly prolonging the time until graft rejection. Earlier studies by Pruitt and Bollinger (1991) used a similar model of a presensitized rat allograft to show that administration of sCR1 alone to inactivate complement resulted in increased time before graft rejection.

Sims et al. (U.S. Patent 5,135,916) suggest using inhibitors of complement, e.g., CD59 or antibodies against C7 or C9 to block the formation of the C5b-9 complex, to treat the vascular endothelium of organs and tissues to be transplanted. This would prevent the C5b-9 initiated cell necrosis. The C5b-9 inactivators would be added to the perfusate or storage medium to protect the vascular lining cells from ongoing complement activation during in vitro storage. Additionally the organ or tissue would be protected from the cytolytic and thrombotic effects arising from complement activation initiated upon transplantation, thereby circumventing complement mediated acute rejection. Sims et al. (U.S. Patent 5,573,940 and U.S. Patent 6,100,443) also teach a method of expressing CD59 in the transplanted tissue or organ to protect the transplanted organ from rejection. This can be accomplished by transfecting the cells being transplanted.

Although the several drugs developed to date in combination with methods of prescreening donors and recipients to match the donor allograft to the recipient have over time increased the average length of time of survival of allografts, many allografts are nonetheless rejected during the life-time of the recipient. In general, the prior art advances have mainly been directed to overcoming acute graft rejection. Further, the role of activated terminal complement components in antibody-mediated allograft rejection has not been examined using inhibitors that specifically target the complement cascade at the C5 protein level. The methods described herein and as exemplified in the Examples advance the allotransplant art by inhibiting chronic rejection of allografts, in particular, allografts in a presensitized recipient. New methods are presented for further prolonging allograft survival by using a proper combination of immunosuppressive drugs in combination with a chronic administration of a complement inhibitor.

Samsung Bioepis Exhibit No. 1002
Page 1012

Methods and Uses

The methods disclosed herein are used to prolong allograft survival. The methods generally include administering an inhibitor of complement activity in combination with one or more immunosuppressants.

Suitable complement inhibitors are known to those of skill in the art. Antibodies can be made to individual components of activated complement, e.g., antibodies to C5a, C7, C9, etc. (see, e.g., U.S. Patent 6,534,058; published U.S. patent application US 2003/0129187; and U.S. Patent 5,660,825). Proteins are known which inhibit complement-mediated lysis, including CD59, CD55, CD46 and other inhibitors of C8 and C9 (see, e.g., U.S. Patent 6,100,443). U.S. Patent 6,355,245 teaches an antibody which binds to C5 and prevents it from being cleaved into C5a and C5b thereby preventing the formation not only of C5a but also the C5b-9 complex. Proteins known as complement receptors and which bind complement are also known (see, Published PCT Patent Application WO 92/10205 and U.S. Patent 6,057,131). Use of soluble forms of complement receptors, e.g., soluble CR1, can inhibit the consequences of complement activation such as neutrophil oxidative burst, complement mediated hemolysis, and C3a and C5a production. Those of skill in the art recognize the above as some, but not all, of the known methods of inhibiting complement and its activation.

Suitable immunosuppressants include, but are not limited to, ATG or ALG, OKT3, daclizumab, basiliximab, corticosteroids, 15-deoxyspergualin, cyclosporins, tacrolimus, azathioprine, methotrexate, mycophenolate mofetil, 6-mercaptopurine, bredinin, brequinar, leflunamide, cyclophosphamide, sirolimus, anti-CD4 monoclonal antibodies, CTLA4-Ig, anti-CD154 monoclonal antibodies, anti-LFA1 monoclonal antibodies, anti-LFA-3 monoclonal antibodies, anti-CD2 monoclonal antibodies, and anti-CD45.

An allograft can include a transplanted organ, part of an organ, tissue or cell. These include, but are not limited to, heart, kidney, lung, pancreas, liver, vascular tissue, eye, cornea, lens, skin, bone marrow, muscle, connective tissue, gastrointestinal tissue, nervous tissue, bone, stem cells, islets, cartilage, hepatocytes, and hematopoietic cells.

At least part of the reason for the failure of allografts is that one response by the recipient of an allograft is the activation of complement. This results in the formation of C5a and C5b-9 which are potent proinflammatory molecules which aid in causing graft failure. Without wishing to be bound by any proposed theory, Applicants theorized that inhibiting the

Samsung Bioepis Exhibit No. 1002
Page 1013

formation of C5a and C5b-9 or inhibiting C5a and C5b-9 which was present would aid in preventing graft failure. Furthermore, it was theorized that so long as the allograft is present, the recipient will continue to attempt to mount an immune response against the graft, and this response will include attempts to produce C5a and C5b-9. If not prevented, this complement response will lead to acute vascular rejection in the short term and could contribute to chronic graft rejection in the long term. Prior art methods of using inhibitors of complement activity were limited to administering these inhibitors only at the time of transplant. This was helpful in preventing acute rejection, but as the results disclosed herein illustrate, improved results are obtained by administration of such inhibitors for a longer term. This long-term administration aids in preventing a chronic rejection of the allograft as opposed to only aiding in preventing an acute rejection. The result is a longer term survival of the allograft as compared to either not administering an inhibitor of complement activity or administering such an inhibitor only at the time of transplant of the allograft. Although very commonly it is desirable that the allograft will survive for the remaining lifetime of the recipient, there are times when the allograft is needed only for a shorter length of time, e.g., a bridge organ to bridge the time until the recipient's own organ can recover on its own, at which time the allograft will no longer be needed. The length of time such a graft will be needed will vary, but will usually be longer than the time at which acute rejection would occur and may be long enough for chronic rejection to occur. This period of desired survival for a bridge graft may be several months, e.g., six months.

To prove that long-term inhibition of complement activity will prolong allograft survival, experiments were performed in which complement activation was inhibited in a chronic fashion and not merely at the time of transplant. Chronic treatment means treatment during an extended period up to the lifetime of the allograft. This can be daily treatment but is not limited to daily treatment. Chronic treatment will maintain an effective amount of the drug in the allograft recipient. For example, a preferred method is to include the anti-C5 monoclonal antibody eculizumab in the treatment. In studies of persons suffering from paroxysmal nocturnal hemoglobinuria (PNH), eculizumab has been administered at a dose of 900 mg/patient once every 12-14 days. This dosing has been found to completely and consistently block terminal complement activity and has greatly inhibited the symptoms of PNH (Hillmen et al., 2004). The administered dose is able to block the effects of complement for approximately two weeks before the eculizumab is inactivated or removed from the body. Therefore, a chronic treatment of eculizumab may be, e.g., the administration

Samsung Bioepis Exhibit No. 1002
Page 1014

of 900 mg to the allograft recipient once every two weeks for the remaining life-time of the patient. Similarly, other drugs can be delivered chronically as needed, whether this is on a daily basis or another schedule is required to maintain an effective amount of the drug in the allograft recipient. Because it is well known that graft rejection can be caused by more than just complement activation, e.g., by T cell activity, the experiments included immunosuppressants such as cyclosporin to further aid in preventing graft rejection.

A preferred method of inhibiting complement activity is to use a monoclonal antibody which binds to complement C5 and prevents C5 from being cleaved. This prevents the formation of both C5a and C5b-9 while at the same time allowing the formation of C3a and C3b which are beneficial to the recipient. Such antibodies that are specific to human complement are known (U.S. Patent 6,355,245). These antibodies disclosed in U.S. Patent 6,355,245 include both a whole or full-length antibody (now named eculizumab) and a single-chain antibody (now named pexelizumab). A similar antibody against mouse C5 is called BB5.1 (Frei et al., 1987). BB5.1 was utilized in the experiments set forth below. Antibodies to inhibit complement activity need not be monoclonal antibodies. They can be, e.g., polyclonal antibodies. They may additionally be antibody fragments. An antibody fragment includes, but is not limited to, an Fab, F(ab'), $F(ab')_2$, a single-chain antibody, a domain antibody and an Fv. Furthermore, it is well known by those of skill in the art that antibodies can be humanized (Jones et al., 1986), chimerized, or deimmunized. An antibody may also comprise a mutated Fc portion, such that the mutant Fc does not activate complement. The antibodies to be used in the present disclosure may be any of these. It is preferable to use humanized antibodies when the recipient of the allograft is a human.

Administration and Formulations

Administration of the inhibitor of complement activity is performed according to methods known to those of skill in the art. These inhibitors are administered preferably before the time of allograft transplantation or at the time of transplantation with administration continuing in a chronic fashion. These inhibitors can additionally be administered during a rejection episode in the event such an episode does occur.

The present disclosure also provides uses of a drug that inhibits complement activity and an immunosuppressive agent in the manufacture of a medicament or medicament package. Such medicament or medicament package is useful in prolonging allograft survival in a recipient, in particular, chronic survival of the allograft. In preferred embodiments, the

Samsung Bioepis Exhibit No. 1002
Page 1015

medicament or medicament package is formulated and prepared such that it is suitable for chronic administration to the recipient of the allograft, for example, stable formulations are employed. In certain embodiments, the medicament or medicament package is formulated and prepared such that it is suitable for concurrent administration of the drug that inhibits complement activity and the immunosuppressive drug to the recipient of the allograft. In certain embodiments, the medicament or medicament package is formulated and prepared such that it is suitable for sequential (in either order) administration of the drug that inhibits complement activity and the immunosuppressive drug to the recipient of the allograft.

A pharmaceutical package of the present disclosure may comprise a drug that inhibits complement activity and at least one immunosuppressive agent. The pharmaceutical package may further comprise a label for chronic administration. The pharmaceutical package may also comprise a label for self-administration by a patient, for example, a recipient of a transplant graft, or instructions for a caretaker of a recipient of a transplant graft. In certain embodiments, the drug and the agent in the pharmaceutical package are in a formulation or separate formulations that are suitable for chronic administration and/or self-administration.

The present disclosure also provides lyophilized formulations and formulations suitable for injection. Certain embodiments provide a lyophilized antibody formulation comprising an antibody that inhibits complement activity and a lyoprotectant. In preferred embodiments, the antibody formulation is suitable for chronic administration, for example, the antibody formulation stable. Alternative embodiments provide an injection system comprising a syringe; the syringe comprises a cartridge containing an antibody that inhibits complement activity and is in a formulation suitable for injection.

An antibody employed in various embodiments of the present disclosure preferably inhibits the formation of terminal complement or C5a. In certain embodiments, antibody inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment. The whole antibody or antibody fragment may be a human, humanized, chimerized or deimmunized antibody or antibody fragment. In certain embodiments, the whole antibody or antibody fragment may inhibit cleavage of complement C5. In certain embodiments, the antibody fragment is a Fab, an F(ab')2, an Fv, a domain antibody, or a single-chain antibody. In preferred embodiments, the antibody fragment is pexelizumab. In alternative preferred embodiments, the whole antibody is eculizumab.

Samsung Bioepis Exhibit No. 1002
Page 1016

In certain embodiments, a drug, such as an antibody, that inhibits complement activity is present in unit dosage form, which can be particularly suitable for self-administration. Similarly, an immunosuppressive agent of the present disclosure may also be present in unit dosage form. A formulated product of the present disclosure can be included within a container, typically, for example, a vial, cartridge, prefilled syringe or disposable pen. A doser such as the doser device described in U.S. Patent 6,302,855 may also be used, for example, with an injection system of the present disclosure.

A "stable" formulation is one in which the drug (e.g., an antibody) or agent therein essentially retains its physical and chemical stability and integrity upon storage. Various analytical techniques for measuring protein stability are available in the art and are reviewed in Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. Adv. Drug Delivery Rev. 10: 29-90 (1993). Stability can be measured at a selected temperature for a selected time period. For example, the extent of aggregation following lyophilization and storage can be used as an indicator of protein stability. For example, a "stable" formulation may be one wherein less than about 10% and preferably less than about 5% of the protein is present as an aggregate in the formulation. In other embodiments, any increase in aggregate formation following lyophilization and storage of the lyophilized formulation can be determined. For example, a "stable" lyophilized formulation may be one wherein the increase in aggregate in the lyophilized formulation is less than about 5% and preferably less than about 3%, when the lyophilized formulation is stored at 2-8 °C for at least one year. In other embodiments, stability of the protein formulation may be measured using a biological activity assay.

A "reconstituted" formulation is one which has been prepared by dissolving a lyophilized protein formulation in a diluent such that the protein is dispersed in the reconstituted formulation. The reconstituted formulation in suitable for administration (e.g. parenteral administration) to a patient to be treated with the protein of interest and, in certain embodiments of the invention, may be one which is suitable for subcutaneous administration.

An isotonic reconstituted formulation is preferable in certain embodiments. By "isotonic" is meant that the formulation of interest has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 250 to 350 mOsm. Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example.

Samsung Bioepis Exhibit No. 1002
Page 1017

A "lyoprotectant" is a molecule which, when combined with a drug (e.g., antibody) of interest, significantly prevents or reduces chemical and/or physical instability of the drug (e.g., antibody) upon lyophilization and subsequent storage. Exemplary lyoprotectants include sugars such as sucrose or trehalose; an amino acid such as monosodium glutamate or histidine; a methylamine such as betaine; a lyotropic salt such as magnesium sulfate; a polyol such as trihydric or higher sugar alcohols, e.g. glycerin, erythritol, glycerol, arabitol, xylitol, sorbitol, and mannitol; propylene glycol; polyethylene glycol; Pluronics; and combinations thereof. The preferred lyoprotectant is a non-reducing sugar, such as trehalose or sucrose.

The lyoprotectant is added to the pre-lyophilized formulation in a "lyoprotecting amount" which means that, following lyophilization of the drug (e.g., antibody) in the presence of the lyoprotecting amount of the lyoprotectant, the drug (e.g., antibody) essentially retains its physical and chemical stability and integrity upon lyophilization and storage.

The "diluent" of interest herein is one which is pharmaceutically acceptable (safe and non-toxic for administration to a human) and is useful for the preparation of a reconstituted formulation. Exemplary diluents include sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (e.g. phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

A "preservative" is a compound which can be added to the diluent to essentially reduce bacterial action in the reconstituted formulation, thus facilitating the production of a multi-use reconstituted formulation, for example. Examples of potential preservatives include octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride (a mixture of alkylbenzyldimethylammonium chlorides in which the alkyl groups are long-chain compounds), and benzethonium chloride. Other types of preservatives include aromatic alcohols such as phenol, butyl and benzyl alcohol, alkyl parabens such as methyl or propyl parahen, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol.

A "bulking agent" is a compound which adds mass to the lyophilized mixture and contributes to the physical structure of the lyophilized cake (e.g. facilitates the production of an essentially uniform lyophilized cake which maintains an open pore structure). Exemplary bulking agents include mannitol, glycine, polyethylene glycol and xorbitol.

Accordingly, a stable lyophilized antibody formulation can be prepared using a lyoprotectant (preferably a sugar such as sucrose or trehalose), which lyophilized formulation

Samsung Bioepis Exhibit No. 1002
Page 1018

can be reconstituted to generate a stable reconstituted formulation having an antibody concentration which is significantly higher (e.g. from about 2-40 times higher, preferably 3-10 times higher and most preferably 3-6 times higher) than the antibody concentration in the pre-lyophilized formulation. Such high protein concentrations in the reconstituted formulation are considered to be particularly useful where the formulation is intended for subcutaneous administration. Despite the very high protein concentration in the reconstituted formulation, the reconstituted formulation can be stable (i.e. fails to display significant or unacceptable levels of chemical or physical instability of the protein) at 2-8 °C. for at least about 30 days. See U.S. Patent 6,821,515. In certain embodiments, the reconstituted formulation is isotonic.

When reconstituted with a diluent comprising a preservative (such as bacteriostatic water for injection, BWFI), the reconstituted formulation may be used as a multi-use formulation. Such a formulation is useful, for example, where a subject patient requires frequent administrations of the drug or antibody and/or agent to treat a chronic medical condition. The advantage of a multi-use formulation is that it facilitates ease of use for the patient, reduces waste by allowing complete use of vial contents, and results in a significant cost savings for the manufacturer since several doses are packaged in a single vial (lower filling and shipping costs).

The present disclosure also provides a method for preparing a formulation comprising the steps of: (a) lyophilizing a mixture of an antibody and a lyoprotectant; and (b) reconstituting the lyophilized mixture of step (a) in a diluent such that the reconstituted formulation is isotonic and stable.

An article of manufacture is also provided herein which comprises: (a) a container which holds a lyophilized mixture of an antibody and a lyoprotectant; and (b) instructions for reconstituting the lyophilized mixture with a diluent to a desirable antibody concentration in the reconstituted formulation. The article of manufacture may further comprise a second container which holds a diluent (e.g. bacteriostatic water for injection (BWFI) comprising an aromatic alcohol).

An injection system of the present disclosure may employ a medication delivery pen as described in U.S. Patent 5,308,341. Medication delivery pens have been developed to facilitate the self-administration of medication. A medication of the present disclosure can be a drug that inhibits complement activity, for example an antibody specific to complement C5,

Samsung Bioepis Exhibit No. 1002
Page 1019

and/or an immunosuppressive agent. One medication delivery pen includes a vial holder into which a vial of insulin or other medication may be received. The vial holder is an elongate generally tubular structure with proximal and distal ends. The distal end of the vial holder includes mounting means for engaging a double-ended needle cannula. The proximal end also includes mounting means for engaging a pen body which includes a driver and dose setting apparatus. A disposable medication containing vial for use with the prior art vial holder includes a distal end having a pierceable elastomeric septum that can be pierced by one end of a double-ended needle cannula. The proximal end of this vial includes a stopper slidably disposed in fluid tight engagement with the cylindrical wall of the vial. This medication delivery pen is used by inserting the vial of medication into the vial holder. A pen body then is connected to the proximal end of the vial holder. The pen body includes a dose setting apparatus for designating a dose of medication to be delivery by the pen and a driving apparatus for urging the stopper of the vial distally for a distance corresponding to the selected dose.

The user of the pen mounts a double-ended needle cannula to the distal end of the vial holder such that the proximal point of the needle cannula pierces the septum on the vial. The patient then selects a dose and operates the pen to urge the stopper distally to deliver the selected dose. The dose selecting apparatus returns to zero upon injection of the selected dose. The patient then removes and discards the needle cannula, and keeps the prior art medication delivery pen in a convenient location for the next required medication administration. The medication in the vial will become exhausted after several such administrations of medication. The patient then separates the vial holder from the pen body. The empty vial may then be removed and discarded. A new vial can be inserted into the vial holder, and the vial holder and pen body can be reassembled and used as explained above.

Accordingly, a medication delivery pen generally has a drive mechanism for accurate dosing and ease of use. A dosage mechanism such as a rotatable knob allows the user to accurately adjust the amount of medication that will be injected by the pen from a prepackaged vial of medication. To inject the dose of medication, the user inserts the needle under the skin and depresses the knob once as far as it will depress. The pen may be an entirely mechanical device or it may be combined with electronic circuitry to accurately set and/or indicate the dosage of medication that is injected into the user. See U.S. Patent 6,192,891.

Samsung Bioepis Exhibit No. 1002
Page 1020

The present disclosure also presents controlled-release or extended-release formulations suitable for chronic and/or self-administration of a medication.

The various formulations can be administered to a patient in need of treatment (e.g., a recipient of an allograft) with the medication (e.g., an antibody of the present disclosure and at least one immunosuppressive agent) by intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

In certain embodiments, a formulation is administered to the patient by subcutaneous (i.e. beneath the skin) administration. For such purposes, the formulation may be injected using a syringe. However, other devices for administration of the formulation are available such as injection devices (e.g. the Inject-ease® and Genject® devices); injector pens (such as the GenPen®); needleless devices (e.g. MediJector® and BioJector®); and subcutaneous patch delivery systems.

The present methods and uses are described with reference to the following Examples, which are offered by way of illustration and are not intended to limit the disclosure in any manner. Standard techniques well known in the art or the techniques specifically described below are utilized. The following abbreviations are used herein: ABMR, antibody-mediated rejection; ACHR, accelerated humoral rejection; ACR, acute cellular rejection; AVR, acute vascular rejection; CsA, cyclosporin; CyP, cyclophosphamide; HAR, hyperacute rejection; MCP-1, monocyte chemotactic protein 1; MST, mean survival time; POD, postoperative day.

Example 1

Methods

Animals and Immunosuppressive Drugs Male adult C3H (H-2^k) mice and BALB/c (H-2^d) mice (Jackson Labs, Bar Harbor, Maine) weighing 25-30 g were chosen as donors and recipients, respectively. In the groups receiving immunosuppression, the recipients were injected with CsA (15 mg/kg/day, s.c., daily from day 0 to endpoint rejection or until day 100), or with CyP (40 mg/kg/day, i.v., on day 0 and 1), or with anti-C5 mAb (clone BB5.1, Alexion Pharmaceuticals Inc., 40 mg/kg/day, i.p., day 0–2, followed by twice a week, day 0-60). Animals were housed under conventional conditions at the Animal Care Facility,

Samsung Bioepis Exhibit No. 1002
Page 1021

University of Western Ontario, and were cared for in accordance with the guidelines established by the Canadian Council on Animal Care. Olfert et al., 1993.

Skin Presensitization Full-thickness skin grafts taken from C3H donors were cut into square pieces of 1×1 cm^2 and transplanted onto the back of the BALB/c recipients' thorax one week prior to heart transplantation from the same donors. Rejection was defined as complete necrosis of the skin grafts.

Abdominal and Cervical Cardiac Transplantation Seven days after skin presensitization, C3H mouse hearts were transplanted into the abdomen of presensitized BALB/c recipients by anastomosing the donor aorta and recipient aorta, and the donor pulmonary artery and recipient inferior vena cava. In the groups with re-transplantation, second heart grafts harvested from either naive C3H mice or long-term surviving presensitized BALB/c recipients were transplanted into the cervical area of the recipients carrying a long-term surviving first abdominal heart graft by anastomosing the donor aorta and recipient carotid artery, and the donor pulmonary artery and recipient external jugular vein (end-to-side). The heart grafts were monitored daily until rejection unless otherwise indicated and rejection was defined as complete cessation of pulsation.

Experimental Groups Presensitized recipients were randomly assigned to eight groups, each consisting of eight animals: Group 1, mice with no treatment; Group 2, mice treated with CsA; Group 3, mice treated with CyP; Group 4, mice treated with CsA plus CyP; Group 5, mice treated with anti-C5 mAb; Group 6, mice treated with anti-C5 mAb plus CsA; Group 7, mice treated with anti-C5 mAb plus CyP; Group 8, mice treated with anti-C5 mAb in combination of CsA and CyP. When cardiac impulses were no longer palpable or at POD100, the grafts were removed for routine histology, immunohistochemistry and western blot analysis, serum samples were collected for flow cytometric analysis and complement hemolytic assay. Five additional animals were placed and sacrificed in groups 6 and 8 on POD3 (MST for groups 1-5, 7) to allow for comparisons at a uniform time point. Serum samples were also collected on POD 11, 21, 28 and 60 in Group 8 for detecting the sequential changes of anti-donor antibody levels and complement activity. In addition, when triple therapy treated presensitized recipients carried a first heart graft for 100 days, they were re-transplanted with a second heart. A naive C3H heart or a 100-day surviving C3H heart from another presensitized BALB/c recipient was used as the second heart. Eight animals were included in each re-transplant group.

Samsung Bioepis Exhibit No. 1002
Page 1022

Graft Histology Tissue samples were fixed in 10% buffered formaldehyde. Specimens were then embedded in paraffin, and sectioned for H&E staining. The microscopic sections were examined in a blinded fashion for severity of rejection by a pathologist. Criteria for graft rejection included the presence of vasculitis, thrombosis, hemorrhage and lymphocyte infiltration. These changes were scored as: 0, no change; 1, minimum change; 2, mild change; 3, moderate change; or 4, marked change.

Immunohistochemistry Four micrometer sections were cut from tissue samples embedded in Tissue-Tek O.C.T gel (Optimum Cutting Temperature, Skura Finetek, Torrance, CA) mounted on gelatin-coated glass microscope slides and stained by a standard indirect avidin-biotin immunoperoxidase staining method using an Elite Vectastain ABC kit (Vector Laboratories Inc., Burlingame, CA). Specimens were stained for $CD4^+$ and $CD8^+$ cells with biotin-conjugated rat anti-mouse CD4 mAb (clone YTS 191.1.2, Cedarlane Laboratories Ltd., Hornby, Ontario, Canada) and biotin-conjugated rat anti-mouse CD8 mAb (clone 53-6.7, Pharmingen, Franklin Lakes, NJ), respectively. Intragraft monocyte/macrophage infiltration was detected by staining with biotin-conjugated rat anti-mouse Mac-1 mAb (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada). Mouse IgG and IgM deposition in grafts was detected using biotin-conjugated goat anti-mouse-IgG and goat anti-mouse-IgM (Cedarlane). For identification of complement deposition, sections were serially incubated with goat anti-C3 or anti-C5 polyclonal Abs (Quidel, San Diego, CA), biotinylated rabbit anti-goat IgG (Vector Laboratories), and HRP-conjugated-streptavidin (Zymed Laboratories, South San Francisco, CA). Slides were washed with phosphate-buffered saline between steps, and examined under light microscopy. Negative controls were performed by omitting the primary antibodies. The immunostaining was scored in five high-power fields of each section, and five independent experiments were performed. The sections of immunoperoxidase staining were graded from 0 to 4+ according to the staining intensity: 0, negative; 1+, equivocal; 2+, weak staining; 3+, moderate staining; and 4+, very intensive staining.

Flow Cytometry The circulating anti-donor specific IgG and IgM antibodies were evaluated in the recipient serum by FACScan flow cytometry (Becton Dickinson, Mountain View, CA). Glotz et al., (1993); Tyan et al. (1994). Briefly, C3H mouse splenocytes were isolated and incubated at 37 °C for 30 minutes with serum from naive control and experimental groups. To stain for total IgG, IgG1, IgG2a, IgG2b and IgM, the cells were washed and incubated with FITC-conjugated goat antibody specific for the Fc portion of mouse IgG or with

Samsung Bioepis Exhibit No. 1002
Page 1023

phycoerythrin-conjugated goat antibody specific for the μ-chain of mouse IgM (Jackson ImmunoResearch Laboratories, West Grove, PA), or with FITC-conjugated goat anti-mouse IgG1 (CALTAG Laboratories, Burlingame, CA), or with FITC-conjugated goat anti-mouse IgG2a (CALTAG), or with FITC-conjugated goat anti-mouse IgG2b (CALTAG). After 1 hour of staining at 4 °C, the cells were washed with PBS, resuspended at 5×10^6/mL, and analyzed by flow cytometry for mean channel fluorescence intensity, which represents the antibody-binding reactivity.

Complement Hemolytic Assay The purified anti-C5 mAb was serially diluted twofold (175-0.1 μg/ml) in GVB^{2+} buffer (gelatin Veronal-buffered saline: 0.1% gelatin, 141 mM NaCl, 0.5 mM $MgCl_2$, 0.15 mM $CaCl_2$, and 1.8 mM sodium barbital) and added in triplicate (50 μl/well) to a 96-well plate. BALB/c mouse serum was diluted to 40% v/v with GVB^{2+} buffer and added (50 μl/ml) to the rows of the same 96-well plate such that the final concentration of BALB/c mouse serum in each well was 20%. The plate was then incubated at room temperature for approximately 30 min while chicken erythrocytes were prepared. Chicken erythrocytes were washed 5×1 ml with GVB^{2+} buffer and resuspended to a final concentration of 5×10^7/ml in GVB^{2+}. Four milliliters of the chicken erythrocytes were sensitized by adding anti-chicken RBC polyclonal antibody (Intercell Technologies, Hopewell, NJ, 0.1% v/v) and the cells were incubated at 4 °C for 15 min with frequent vortexing. The cells were then washed 2×1 ml with GVB^{2+} and resuspended to a final volume of 2.4 ml in GVB^{2+}. The chicken erythrocytes (30 μl/well, 2.5×10^6 cells) were added to the plate containing serum and anti-C5 mAb as described above, mixed well, and incubated at 37 °C for 30 min. The plate was then centrifuged at 1000×g for 2 min, and 85 μl of the supernatant was transferred to a new 96-well microtiter plate. The plate was read at OD 415 nm using a microplate reader and the percentage of hemolysis was determined using this formula:

$$\%\ \text{hemolysis} = 100 \times \frac{(\text{OD sample}) - (\text{OD } GVB^{2+} \text{ control})}{(\text{OD 100\% lysed control}) - (\text{OD } GVB^{2+} \text{ control})}$$

with 100% lysed control obtained by the addition of 100 μl GVB^{2+} containing 0.1% NP-40 to the 30 μg/ml of chicken erythrocytes as prepared above.

Samsung Bioepis Exhibit No. 1002
Page 1024

Western Blot Analysis Sonication of frozen heart samples was performed in RIPA lysis buffer (Santa Cruz Biotechnology, Inc.) at 4 °C for 1 minute at 10-second intervals, followed by microcentrifugation at 13,000 rpm for 10 minutes at 4 °C. Clarified supernatants were immediately quantitated in triplicate for protein content using Detergent-compatible protein assay kit (BIO-RAD). Heart lysates (10μg protein/well) were separated on NuPAGE, 4-12% gradient Bis-Tris gels and MES buffer system (Invitrogen) and transferred to polyvinylidene difluoride (PVDF) membrane (0.45 μm pore size; Invitrogen) using a semi-dry transfer apparatus (BIO-RAD). Membranes were cut appropriately at the correct molecular weights to allow the development of the blots with two different primary antibodies per blot such that each blot was exposed to a test antibody and an internal control antibody to insure equal sample loading. The test primary antibodies including anti-Bcl-2 (N-19) rabbit polyclonal sera (Santa Cruz Biotechnology, Inc.) and anti-Bcl-XS/L (M-125) rabbit polyclonal sera (Santa Cruz Biotechnology, Inc.) were used to detect intragraft expression of Bcl-2 and Bcl-xl proteins. Anti-calsequestrin rabbit polyclonal sera (Calbiochem) were used as internal control primary antibody (Kobayashi et al., 1999). Detection of primary antibody binding was performed as previously described (Arp et al., 1996) by exposing washed incubated blots to a polyclonal goat anti-rabbit IgG fraction conjugated to horseradish peroxidase (HRP; Roche Laboratories) and then appropriately developed by exposure to enhance chemiluminescence for HRP-conjugated antibodies (Roche Laboratories).

Statistical Analysis The data were reported as the mean ± SD. Allograft survival among experimental groups was compared using the rank-log test. Histological and immunohistological findings were analyzed using the Mann-Whitney U test. Flow cytometric data and western blot data were analyzed using one-way ANOVA. Differences with p values less than 0.05 were considered significant.

Example 2

Presensitization with C3H donor skin graft induces antibody-mediated ACHR in heart allografts of BALB/c recipients.

To develop a suitable small animal model that mimics presensitized patients in the clinic and to study ABMR, a novel, fully MHC-mismatched mouse ABMR model has been developed through presensitization of mouse recipients. In this model, BALB/c recipients were presensitized with C3H donor skin grafts one week prior to heart transplantation from the same donor. Seven days after donor skin presensitization, serum level of anti-donor IgG,

Samsung Bioepis Exhibit No. 1002
Page 1025

but not IgM antibody was markedly elevated and reached to a peak level in the presensitized BALB/c recipients (Figure 1A). Heart transplantation from same donor was then performed in these highly sensitized recipients. Without immunosuppression, C3H heart grafts were rapidly rejected in 3.1 ± 0.4 days by ACHR, characterized by severe thrombosis, hemorrhage and infarction (Figure 1B-a). In contrast, same heart grafts in unsensitized BALB/c recipients (with mean survival time, MST of 8.2 ± 0.8 days) show the normal histology on post-operative day (POD) 3 (Figure 1B-b). When compared to unsensitized BALB/c recipients at the same day, heart grafts in presensitized animals revealed massive IgG antibody and complement (C3 and C5) deposition, but minimal $CD4^+$ and $CD8^+$ cell infiltration (Table 1). Furthermore, circulating anti-donor IgG levels in presensitized recipients were significantly higher than those of unsensitized same recipients receiving a heart graft on POD3 ($P< 0.01$, Figure 1C). However, anti-donor IgM remained at very low levels both in circulation (Figure 1C) and in heart grafts (Table 1) and it showed no significant difference between unsensitized and presensitized recipients. In addition, normal levels of complement hemolytic activity were shown in both presensitized and unsensitized heart recipients without treatment (Figure 1D). These data indicate that this is an ideal transplant model to study ABMR in presensitized recipients in which complement plays an important role in the pathogenesis.

Table 1.

Comparison of immunohistological changes of C3H heart allografts in unsensitized and presensitized BALB/c recipients on POD3

Groups	Unsensitized	Presensitized
IgG	1+	4+
IgM	1+	1+
C3	2+	3+
C5	2+	3+
CD4	0	1+
CD8	0	1+

Samsung Bioepis Exhibit No. 1002
Page 1026

Grades for immunoperoxidase staining: 0, negative; 1+, equivocal; 2+, weak; 3+, moderate; 4+, intense.

Anti-C5 mAb in combination with CsA and CyP prevents ABMR and achieves indefinite heart allograft survival in presensitized mouse recipients.

Complement has been shown to play an important role in ABMR. However, the inhibitory effect of functionally blocking terminal complement cascade at the C5 level in highly sensitized recipients is unknown. In the study presented herein, the presensitized model was used to study the efficacy of anti-C5 mAb either alone or combined with CsA and /or CyP in prevention of ABMR. As presented in Figure 2A, treatment with either CsA or CyP or the two drugs in combination did not prevent ABMR and grafts were rejected in 3.0 ± 0.0 days, 3.3 ± 0.5 days and 3.5 ± 0.6 days, respectively with typical pathological features of ACHR including intravascular thrombosis and interstitial hemorrhage (Figure 2B-b, c, d), which were indistinguishable from heart grafts in untreated presensitized BALB/c recipients (Figure 2B-a). Anti-C5 monotherapy or combined with CyP was not able to improve graft survival and heart grafts were rejected by ACHR (Figure 2B-e, f) in 3.5 ± 0.6 days and 3.2 ± 0.4 days, respectively (Figure 2A). Although the combination therapy of anti-C5 mAb and CsA, the protocol capable of inducing long-term heart allograft survival in unsensitized animals, marginally prolonged graft survival in this presensitized model, heart grafts were also rejected by severe humoral rejection with vasculitis, thrombosis, hemorrhage and minimal cell infiltration (Figure 2B-g) in 11.9 ± 1.8 days (Figure 2A). In contrast, triple therapy of anti-C5 mAb in combination of CsA and CyP achieved indefinite heart graft survival over 100 days (Figure 2A) in presensitized animals ($P<0.01$ vs. the animals without treatment or treated with either monotherapy or two drugs in combination) with no evidence of rejection (Figure 2B-h). In this presensitized mouse model, as shown in Table 2, only minor intragraft $CD4^+$ and $CD8^+$ cell infiltration was observed in the recipients that rejected their heart grafts within 3 days. However, the number of these T cells was slightly increased if heart grafts survived longer in anti-C5 mAb plus CsA-treated recipients at the time of rejection (POD11) and in triple therapy-treated recipients at early stages of graft survival (e.g. POD11). Furthermore, with continuous treatment of CsA in the triple therapy group, $CD4^+$ and $CD8^+$ cell infiltration was inhibited in long-term surviving heart grafts on POD60 and 100. In addition, moderate intragraft Mac-1^+ cell infiltration, including monocytes and macrophages, was found in untreated and CsA-, CyP- or CsA plus CyP-treated animals,

Samsung Bioepis Exhibit No. 1002
Page 1027

while the infiltration of these cells was significantly reduced in anti-C5 mAb treated animals (Table 2). These results indicate that functionally blocking anti-C5 mAb enables the use and efficacy of conventional immunosuppressive agents, thereby preventing ABMR and achieving indefinite heart graft survival in presensitized recipients.

Table 2.

Grades for immunoperoxidase staining of heart allografts

in presensitized mouse recipients at necropsy

Groups	Date for sample collection (POD)	C3	C5	**CD4**	CD8	Mac-1	IgG	IgM
Untreated	3	3+	3+	1+	1+	3+	4+	1+
CsA	3	3+	3+	1+	1+	3+	4+	1+
CyP	3	3+	3+	1+	1+	3+	3+	1+
CsA+CyP	3	3+	3+	1+	1+	3+	3+	1+
Anti-C5mAb	3	3+	0	1+	1+	2+	4+	1+
Anti-C5mAb+CsA	11	3+	0	2+	2+	2+	4+	1+
Anti-C5mAb+CyP	3	3+	0	1+	1+	2+	3+	1+
Anti-C5mAb+CsA+CyP	3	3+	0	1+	1+	2+	3+	1+
Anti-C5mAb+CsA+CyP	11	3+	0	2+	2+	1+	3+	1+
Anti-C5mAb+CsA+CyP	60	3+	0	1+	1+	0	2+	1+
Anti-C5mAb+CsA+CyP	100	3+	2+	0	0	0	2+	1+

Grades: 0, negative; 1+, equivocal; 2+, weak; 3+, moderate; 4+, intense.

Anti-C5 mAb completely inhibits total complement hemolytic activity and local C5 deposition in presensitized recipients receiving a heart allograft.

Anti-C5 mAb was previously shown to block the cleavage of complement protein C5 into the proinflammatory molecules C5a and C5b-9 (Kroshus et al., 1995), and to completely and consistently block terminal complement activity in mice (Wang et al., 1999). In the current study, terminal complement activity was measured by assessing the ability of

Samsung Bioepis Exhibit No. 1002
Page 1028

recipient mouse sera to lyse antibody presensitized chicken erythrocytes and was compared at the same time-point (POD3). Treatment of mice with either CsA or CyP or the two drugs in combination had no effect on terminal complement activity, while treatment with anti-C5 mAb either alone or combined with CsA or/and CyP completely inhibited this activity (Figure 3; $P<0.01$, vs. naive and untreated animals, as well as CsA-, CyP-, or CsA plus CyP-treated animals). In addition, sera obtained from anti-C5 mAb treated animals at several earlier time points showed similarly diminished hemolytic activity, suggesting that serum terminal complement was inhibited throughout the treatment period. Furthermore, local C5 deposition in heart grafts was completely prevented in the anti-C5 mAb treated presensitized recipients, but not in untreated, or CsA-, CyP- and CsA plus CyP-treated presensitized animals (Table 2). As predicted, treatment with anti-C5 mAb did not prevent C3 deposition in the grafts (Table 2). These results suggest anti-C5 therapy completely blocks total complement activity after cardiac allografting in highly sensitized recipients.

Long-term surviving heart grafts in presensitized animals are resistant to humoral injury in the presence of low level of anti-donor antibodies and complement- a situation of accommodation.

To further investigate the role of anti-C5 mAb in humoral rejection, anti-donor alloantibody levels were measured in recipient sera by flow cytometry and intragraft antibody deposition by using immunostaining techniques in different groups. Figure 4A shows that on POD3 untreated presensitized BALB/c recipients had high levels of circulating anti-donor IgG antibodies. When presensitized recipients receiving either monotherapy or two drugs in combination, CsA and/or CyP partially down-regulated circulating anti-donor IgG levels, while treatment with anti-C5 mAb either alone or combined with CsA or CyP did not further affect anti-donor antibody levels at the same day. In contrast, with triple therapy of anti-C5 mAb, CsA and CyP, a high level of circulating anti-donor IgG was gradually downregulated and reached a low level on POD60, thereafter remaining at this level until day 100 (Figure 4B). Similar to levels of circulating antibodies in the different treatment groups, Table 2 shows that strong deposition of anti-mouse IgG was present in the rapidly rejected heart grafts of presensitized animals with no treatment or treated with monotherapy or two drugs in combination therapy. Interestingly, with triple therapy, IgG antibody deposition was gradually attenuated to a mild level in the long-term surviving heart grafts on POD 100 (Figure 4C-a, Table 2). In this model, IgM remained at very low levels in either circulation (Figure 4A, B) or transplanted heart grafts (Figure 4C-b, Table 2) in presensitized recipients

Samsung Bioepis Exhibit No. 1002
Page 1029

with or without treatment. In addition, treatment with anti-C5 mAb eliminated complement activity to an undetectable level until day 60, followed by a progressive recovery to predepletion levels on POD 100 after discontinuation of anti-C5 therapy in presensitized mouse recipients receiving triple therapy (Figure 4D). Furthermore, intragraft C5 deposition was also detected in 100-day surviving presensitized animals (Table 2). These data demonstrate that ongoing transplant accommodation occurs in triple therapy treated presensitized recipients despite the presence of anti-graft antibodies and complement activation.

Anti-C5 mAb in combination with CsA and CyP reduces the IgG1/IgG2a ratio and leads to a shift in IgG subclass to IgG2b in recipients with accommodated grafts.

To determine whether anti-C5 mAb-based triple therapy would induce a shift in IgG subclass, which may be associated with accommodation, serum levels of anti-donor IgG subclasses of IgG1, IgG2a and IgG2b were compared between untreated recipients and the recipients with accommodated heart graft. Sera from untreated recipients contained predominant IgG1 isotype, indicated by a high ratio of IgG1/IgG2a (Figure 5A). In contrast, a significant reduction in the ratio of IgG1/IgG2a was observed in the recipients carrying accommodated grafts (Figure 5A, $P<0.01$). Furthermore, presensitized recipients with the accommodated heart grafts displayed an increased level of anti-donor IgG2b as compared to the same recipients with rejected grafts (Figure 5B, $P<0.01$). In addition, the pattern of IgG isotypes in the recipients treated with either monotherapy or two drugs in combination is indistinguishable from that of untreated animals. These data indicate that anti-donor IgG1 isotype may be associated with graft rejection, while production of anti-donor IgG2b subclass may function as a protective antibody and plays an important role in the induction of accommodation.

Anti-C5 mAb in combination with CsA and CyP induces intragraft Bcl-2 and Bcl-xl expression in highly sensitized mouse recipients.

To determine whether a causal relationship exists between intragraft expression of protective proteins and graft resistance to humoral injury in this model, western blot analysis was employed to detect proteins of interest in heart graft tissues from highly sensitized mouse recipients. Long-term surviving heart grafts were found to express high levels of Bcl-2 and Bcl-xl proteins on POD100, and these proteins were detected as early as 12 days after heart transplantation in highly sensitized recipients receiving anti-C5 mAb-based triple therapy

Samsung Bioepis Exhibit No. 1002
Page 1030

(Figure 6). In contrast, there were no Bcl-2 and Bcl-xl proteins expressed on heart grafts of untreated animals (Figure 6) or animals treated with either monotherapy or two drugs in combination therapy. This result suggests that graft resistance to humoral injury in indefinite surviving animals is associated with the protection provided by Bcl-2 and Bcl-xl proteins in this presensitized model.

Presensitized recipients with an accommodating first heart graft accept a second accommodated heart graft but reject a second naive heart graft from the same donors.

The ability of accommodated grafts to resist rejection has not been tested directly under pathophysiological conditions where naive grafts undergo rejection following allotransplantation. In this model, to determine whether presensitized recipients with an accommodating first heart graft will accept a second accommodated graft but reject a second naive graft, we performed re-transplantation scenarios. After the accommodated C3H heart grafts have survived to the 100-day point, the time at which low levels of alloantibodies were detected (Figure 4B) and complement activity has returned to pretreatment levels (Figure 4D), in presensitized BALB/c recipient treated with anti-C5 mAb-based triple therapy, these recipients received a second heart graft. Specifically, either a naive (Figure 7A) or a 100-day accommodated C3H heart (Figure 7B) from another presensitized BALB/c recipient was transplanted into the neck of the presensitized recipients carrying an accommodating first C3H heart. These recipients rejected a second naive heart at 6.6 ± 1.1 days (Figure 8A) with severe AVR (Figure 8B-a) while the first heart continued to survive. In contrast, when the accommodated hearts that had been already surviving for 100 days in different presensitized mice were used as second grafts, these grafts were accepted by the presensitized recipients carrying an accommodating first heart graft (Figure 8A). There was no sign of rejection in those accommodated second heart grafts 90 days after second transplantation (Figure 8B-b). These data indicate that accommodated grafts become resistant to the effects of anti-donor antibodies and complement that normally mediate allograft rejection in these presensitized recipients. Furthermore, the fact that the host of the accommodated graft rejected a new graft suggests that accommodation involves changes to the graft.

Presensitized recipients being treated with CsA reject accommodated heart grafts.

Another re-transplantation was performed to determine whether accommodation in this presensitized model would be caused by the changes in the grafts and/or the recipients. Specifically, after C3H heart grafts have been accommodated in presensitized BALB/c mice

Samsung Bioepis Exhibit No. 1002
Page 1031

for 100 days, the accommodated heart graft will then be re-transplanted into a second presensitized BALB/c recipient being treated with CsA alone (Figure 7C), a therapy that can prevent cellular rejection but cannot prevent accelerated humoral rejection of a fresh C3H heart in presensitized recipients. The accommodated C3H heart grafts were rapidly rejected in CsA treated presensitized BALB/c recipients. After re-transplantation, the pathology in accommodated heart grafts was changed from normal (Figure 9A) to severe ACHR with massive interstitial hemorrhage but few cell infiltrates (Figure 9B). In addition, high levels of anti-donor IgG and normal levels of complement hemolytic activity in these recipients receiving an accommodated C3H heart were similar to those of CsA treated presensitized recipients receiving a naive C3H heart. This result further indicates that accommodation induced by anti-C5 mAb-based triple therapy can originate from mechanisms involving changes not only to the graft, but also to the recipient.

Example 3

Acute Vascular Rejection in a Heart Transplantation Model

Experiments were performed to determine whether inclusion of an inhibitor of formation of terminal complement would attenuate acute vascular rejection and whether the use of such an inhibitor in conjunction with an immunosuppressant would achieve long-term allograft survival. In this set of experiments an anti-C5 monoclonal antibody was used in conjunction with cyclosporin. The model used was an allograft heterotopic heart transplant from C3H mice into BALB/c mice. This model is a stringent acute vascular rejection model with the C3H and BALB/c mice being strongly MHC mismatched. The transplantations and other methods were performed as described in Wang et al. (2003).

Heterotopic Cardiac Transplantation

Intra-abdominal heterotopic cardiac transplantation was performed as previously described by Wang et al. (2003). Briefly, a median sternotomy was performed on the donor, and the heart graft was slowly perfused in situ with 1.0 ml of cold heparinized Ringer's lactate solution through the inferior vena cava and aorta before the superior vena cava and pulmonary veins were ligated and divided. The ascending aorta and pulmonary artery were transected, and the graft was removed from the donor. The graft was then revascularized with end-to-side anastomoses between the donor's pulmonary artery and the recipient's inferior vena cava as well as the donor's aorta and the recipient's abdominal aorta using 11-0 nylon suture. The beating of the grafted heart was monitored daily by direct abdominal palpation.

Samsung Bioepis Exhibit No. 1002
Page 1032

The degree of pulsation was scored as: A, beating strongly; B, noticeable decline in the intensity of pulsation; or C, complete cessation of cardiac impulses. When cardiac impulses were no longer palpable, the graft was removed for routine histology. In certain instances, mice in which the graft was still functioning were sacrificed to perform histology.

Results

Mice (male 8-12 week old mice weighing 25 – 30 g) were split into six experimental groups with six to eight mice per group. Transplant occurred on day 0. Histological changes were checked at the endpoint (the endpoint being graft failure) or in some cases a mouse was sacrificed prior to graft failure. The dosage of BB5.1 which was administered (40 mg/kg body weight three times per week) was known from prior studies to completely inhibit terminal complement activity.

Group 1' (control) - mice were administered 0.75 mL of saline intraperitoneally on days -1, 0, 1 and 2. Subsequently these mice were treated with 0.75 mL of saline intraperitoneally three times per week (Monday, Wednesday, Friday) until the endpoint.

Group 2' (cyclosporin A alone) - mice were administered 15 mg/kg body weight of cyclosporin A subcutaneously on a daily basis beginning at day 0 (day of transplant) until the endpoint.

Group 3' (anti-complement antibody alone) - mice were administered the anti-mouse C5 antibody BB5.1 (Frei et al., 1987) at 40 mg/kg body weight intraperitoneally on days -1, 0, 1 and 2 followed by 40 mg/kg body weight administered three times per week (Monday, Wednesday, Friday) until the endpoint.

Group 4' (anti-complement antibody until day 14 post-transplant plus cyclosporin A) - mice were administered the anti-mouse C5 antibody BB5.1 at 40 mg/kg body weight intravenously on days -1 through day 14 and were also administered cyclosporin A at 15 mg/kg of body weight on a daily basis beginning at day 0 until the endpoint. Note that this differs from the other groups in that the BB5.1 was administered intravenously and on a daily basis.

Group 5' (anti-complement antibody until day 28 post-transplant plus cyclosporin A) - mice were administered the anti-mouse C5 antibody BB5.1 at 40 mg/kg body weight intraperitoneally on days -1, 0, 1 and 2 followed by 40 mg/kg body weight administered three times per week (Monday, Wednesday, Friday) until day 28 and were also administered

Samsung Bioepis Exhibit No. 1002
Page 1033

cyclosporin A at 15 mg/kg of body weight on a daily basis beginning at day 0 until the endpoint.

Group 6' (anti-complement antibody chronically until 100 days plus cyclosporin) - mice were administered the anti-mouse C5 antibody BB5.1 at 40 mg/kg body weight intraperitoneally on days -1, 0, 1 and 2 followed by 40 mg/kg body weight administered three times per week (Monday, Wednesday, Friday) until 100 days and were also administered cyclosporin A at 15 mg/kg of body weight on a daily basis beginning at day 0 until 100 days.

The results of this experiment are shown in Tables 3 and 4. Table 3 shows the survival time for the grafts. Table 4 sets forth the histological scores.

Table 3

Allograft Survival

Group (Treatment)	Individual Survival (days)	Mean Survival Time (days)
1'. Saline	8, 8, 8, 8, 8, 9	8.3 ± 0.5
2'. Cyclosporin A	14, 15, 15, 16, 16, 16, 17	15.5 ± 1.1
3'. BB5.1	7, 8, 8, 8, 8, 9	8.0 ± 0.6
4'. BB5.1 until day 14 + cyclosporin A	35, 38, 43, 45, 46, 47	42.3 ± 4.8
5'. BB5.1 until day 28 + cyclosporin A	77, 80, 80, 81, 82	80 ±1.9
6'. BB5.1 until day 100 + cyclosporin A	>100 days (7 mice; one sacrificed for histology)	> 100 days

Samsung Bioepis Exhibit No. 1002
Page 1034

Table 4

Median Scores of Histological Changes of Heart Allografts at Necropsy

Groups	Vasc*	Infarc	Lymph	Throm	Hemo	Fibrin	PMN
1'. Saline (endpoint)	3.0	3.0	1.0	4.0	3.0	3.0	3.0
2'. Cyclosporin A (endpoint)	2.0	1.0	2.0	3.0	2.0	2.0	3.0
3'. BB5.1 (endpoint)	2.0	1.0	2.0	2.0	1.0	0.0	0.0
4'. BB5.1 until day 14 + cyclosporin A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
5'. BB5.1 28 days + Cyclosporin A (post-operative day 8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5'. BB5.1 28 days + Cyclosporin A (endpoint)	0.0	0.0	1.0	1.0	2.0	1.0	0.0
6'. BB5.1 until day 100 + Cyclosporin A (post-operative day 100)	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Median scores: 0 - normal; 1- minimum change; 2 - mild change; 3 - moderate change; 4 - marked change. N/A - not available.

*Vasc - vasculitis; Infar - infarction; Lymph - lymphocyte infiltration; Throm - thrombosis; Hemo - hemorrhage; Fibrin - fibrin deposition; PMN - polymorphonuclear cell infiltrate

The results indicate the synergistic effects of using a complement inhibiting drug in addition to an immunosuppressant. In untreated mice the grafts were rejected in about 8 davs. Use of the immunosuppressant cyclosporin A alone on a daily, chronic basis resulted

Samsung Bioepis Exhibit No. 1002
Page 1035

in an increase in graft survival until approximately 15 days post-transplant. The use of the anti-C5 antibody BB5.1 to inhibit formation of terminal complement had no effect on its own, graft rejection occurring at 8 days post-transplant as in the control group (Group 1'). The combination of BB5.1 through day 28 post-transplant plus cyclosporin A showed a synergistic effect with graft survival being extended until approximately day 80. A more surprising result is that of Group 5' in which BB5.1 and cyclosporin A were each administered chronically post-transplant. In this case the graft survival was for more than 100 days (as much data as presently available). Additionally, the histological results shown in Table 4 indicate that the administration of both BB5.1 and cyclosporin A protected the graft from changes much better than either BB5.1 or cyclosporin A alone, and that the chronic administration of BB5.1 and cyclosporin A protected the graft to such an extent that even at 100 days post-transplant there were no histological changes seen in the engrafted hearts. A survival time of 100 days in these models is considered to be the gold standard. A survival of 100 days in the model is believed to indicate that there will be an indefinite survival of the allograft. When BB5.1 administration was stopped after 28 days, the grafts were protected but they did begin to show some minimal to mild histological changes by about day 80 which was the time at which graft failure occurred.

The Group 4' mice were treated differently in that they were administered BB5.1 on a daily basis by an intravenous administration. These animals became ill, showing weight loss and urine retention and were sacrificed at a time at which the grafted hearts were still beating although their function had declined. This was the first group of mice studied and it is unknown why these ill effects were seen. These ill effects were not seen when the BB5.1 was administered intraperitoneally with a schedule of three times per week. As seen below in Example 4, daily administration of BB5.1 via an intraperitoneal route did not cause ill effects. Also, intravenous administration was not necessarily the cause of the illness in these animals. Intravenous administration of eculizumab (a human equivalent antibody to BB5.1 in that it binds to human C5) has been successfully administered intravenously without ill effects to humans in a study of PNH (Hillmen et al., 2004). Complement inhibitors may be administered by other routes in addition to intravenous and intraperitoneal, with all such routes being well known by those skilled in the art.

Example 4

Samsung Bioepis Exhibit No. 1002
Page 1036

Accelerated Rejection in a Presensitized Heart Transplantation Model

A second set of experiments similar to those of Example 3 was performed but the recipient mouse was presensitized to the donor organ. In these experiments, the presensitization was brought about by prior transplantation of a skin graft. In general, presensitization can occur not only as a result of having received an earlier allograft, but can also be caused by having received multiple blood transfusions or in women who have been pregnant. Besides such presensitization methods, allografts with an ABO mismatch will be rapidly attacked and rejected because of preformed antibodies to the ABO antigens unless steps are taken to prevent such an attack.

Some mice in these studies were administered cyclophosphamide in addition to BB5.1 and/or cyclosporin A. For these experiments BALB/c recipient mice were presensitized with C3H skin grafts one week prior to heart transplantation from the same donor (using the method of Pruitt and Bollinger, 1991). This model is designed to mimic presensitized transplantation in humans, especially in relation to accelerated humoral rejection. Recipient mice were split into eight groups of six to eight mice each. The treatments were as follow.

Group 1" (control) - mice (male 8-12 week old mice weighing 25 – 30 g) were administered 0.75 mL saline intraperitoneally on a daily basis beginning at day -1 and continuing until the endpoint (graft rejection).

Group 2" (cyclosporin A alone) - mice were administered cyclosporin A subcutaneously at a dose of 15 mg/kg body weight beginning on day 0 (day of transplant) until the endpoint.

Group 3" (BB5.1 alone) - mice were administered the anti-mouse complement monoclonal antibody BB5.1 at a dose of 40 mg/kg body weight delivered intraperitoneally on a daily basis beginning at day -1 and continuing until the endpoint.

Group 4" (cyclophosphamide alone) - mice were administered cyclophosphamide intravenously at a dose of 40 mg/kg body weight on each of days 0 and 1.

Group 5" (BB5.1 plus cyclosporin A) - mice were administered BB5.1 intraperitoneally at a dose of 40 mg/kg body weight on a daily basis beginning at day -1 and continuing until the endpoint. These mice were additionally administered cyclosporin A subcutaneously at a dose of 15 mg/kg body weight on a daily basis from day 0 until the endpoint.

Samsung Bioepis Exhibit No. 1002
Page 1037

Group 6" (BB5.1 plus cyclophosphamide) - mice were administered BB5.1 intraperitoneally at a dose of 40 mg/kg body weight on a daily basis beginning at day -1 and continuing until the endpoint. These mice were additionally administered cyclophosphamide intravenously at a dose of 40 mg/kg body weight on each of days 0 and 1.

Group 7" (cyclosporin A plus cyclophosphamide) - mice were administered cyclosporin A subcutaneously at a dose of 15 mg/kg body weight on a daily basis from day 0 until the endpoint. These mice were additionally administered cyclophosphamide intravenously at a dose of 40 mg/kg body weight on each of days 0 and 1.

Group 8" (BB5.1 plus cyclosporin A plus cyclophosphamide) - mice were administered BB5.1 intraperitoneally at a dose of 40 mg/kg body weight on a daily basis beginning at day -1 and continuing until 100 days. These mice were also administered cyclosporin A subcutaneously at a dose of 15 mg/kg body weight on a daily basis from day 0 until 100 days. These mice were additionally administered cyclophosphamide intravenously at a dose of 40 mg/kg body weight on each of days 0 and 1. Two mice in this group were sacrificed at day 60 for histological studies (no rejection had yet occurred) and the four remaining mice still had not rejected their grafts by day 100.

Additionally a control group of mice which was not presensitized and received only the saline treatment as for Group 1" was tested.

The results of these experiments are shown in Tables 5 and 6. Table 5 lists survival times for the grafts and Table 6 summarizes the histological results.

Samsung Bioepis Exhibit No. 1002
Page 1038

Table 5

Allograft Survival

Groups (Treatment)	Individual survival (days)	Mean survival time (days)
No presensitization	8, 8, 8, 8, 8, 9	8.3 ± 0.5*
1". One skin presensitization	3, 3, 3, 3, 3, 3, 3, 4	3.1 ± 0.4
2". Cyclosporin A	3, 3, 3, 3	3.0 ± 0.0
3". BB5.1	3, 3, 4, 4	3.5 ± 0.6
4". Cyclophosphamide	3, 3, 3, 4	3.3 ± 0.5
5". BB5.1 + Cyclosporin A	10, 10, 11, 11, 12, 12, 14, 15	11.9 ± 1.8**
6". BB5.1 + Cyclophosphamide	3, 3, 3, 3, 3, 4	3.2 ± 0.4
7". Cyclosporin A + Cyclophosphamide	3, 3, 3, 4, 4, 4	3.5 ± 0.6
8". BB5.1 + Cyclosporin A + Cyclophosphamide	> 100 days (4 mice)	> 100***

*P < 0.01 group 1" vs. no presensitization

**P < 0.01 group 5" vs. groups 1"-4" and 6"-7".

***P<0.01 group 8" vs. groups 1"-7".

Samsung Bioepis Exhibit No. 1002
Page 1039

Table 6

Median Scores of Histological Changes of Heart Allografts at Necropsy

Groups	Vasc*	Infar	Lymph	Throm	Hemo	Fibrin	PMN
No presensitization (endpoint)	3.0	3.0	1.0	4.0	3.0	3.0	3.0
1". One skin presensitization (endpoint)	0.0	4.0	1.0	4.0	3.0	0.0	0.0
2". Cyclosporin A (endpoint)	0.0	4.0	0.0	3.0	3.0	N/A	N/A
3". BB5.1 (endpoint)	2.0	2.0	2.0	2.0	3.0	N/A	N/A
4". Cyclophosphamide (endpoint)	2.0	4.0	0.0	3.0	2.0	N/A	N/A
5". BB5.1 + Cyclosporin A (post-operative day 3)	0.0	1.0	1.0	0.0	0.0	0.0	0.0
5". BB5.1 + Cyclosporin A (endpoint)	2.0	2.0	2.0	2.0	2.0	0.0	0.0
6". BB5.1 + Cyclophosphamide (endpoint)	1.0	3.0	0.0	3.0	2.0	N/A	N/A
7". Cyclosporin A + Cyclophosphamide (endpoint)	0.0	1.0	1.0	2.0	3.0	N/A	N/A
8". BB5.1 + Cyclosporin A + Cyclophosphamide (post-operative day 3)	0.0	0.0	1.0	0.0	0.0	N/A	N/A
8. BB5.1 + Cyclosporin A + Cyclophosphamide (post-operative day 12)	0.0	0.0	1.0	0.0	0.0	N/A	N/A
8". BB5.1 + Cyclosporin A + Cyclophosphamide (post-	0.0	0.0	1.0	0.0	0.0	N/A	N/A

Samsung Bioepis Exhibit No. 1002
Page 1040

operative day 60)							
8". BB5.1 + Cyclosporin A + Cyclophosphamide (post-operative day 100)	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Median scores: 0 - normal; 1- minimum change; 2 - mild change; 3 - moderate change; 4 - marked change. N/A - not available.

*Vasc - vasculitis; Infar - infarction; Lymph - lymphocyte infiltration; Throm - thrombosis; Hemo - hemorrhage; Fibrin - fibrin deposition; PMN - polymorphonuclear cell infiltrate

The results shown in Table 5 indicate a difference between the presensitized mouse model and the nonpresensitized mouse model as used in Example 3. The results indicate that in the absence of presensitization, grafts are rejected in approximately 8 days in the absence of treatment with any drugs. Presensitizing the animals causes a more rapid rejection, the rejection of the graft in the presensitized animals being in approximately 3 days in the absence of any drug treatment. Treatment with either BB5.1, cyclosporin A or cyclophosphamide had no effect upon graft survival, with the grafts being rejected in approximately 3-4 days in each of these groups of animals. The combination of BB5.1 and cyclosporin A showed some effect with rejection occurring about day 12. The combination of BB5.1 and cyclophosphamide had no protective effect with rejection occurring about day 3. Similarly the combination of cyclosporin A and cyclophosphamide had essentially no protective effect with rejection occurring at 3-4 days. Very surprisingly, the combination of all three drugs (chronic administration of BB5.1 and cyclosporin plus administration of cyclophosphamide at the time of transplant) showed a highly synergistic effect with all of the mice surviving for more than 100 days. Again, a survival of 100 days in this model is considered to be the gold standard and assumes an indefinite survival.

These results as well as the histological results as shown in Table 6 indicate that the combination of chronic treatment with a complement inhibitor and an immunosuppressant such as cyclosporin A in treating a presensitized mouse results in some attenuation of accelerated rejection. Treatment of these animals additionally with cyclophosphamide at the time of transplant and on the first day after transplant results in a much greater time of survival, no rejection having been seen by at least day 100.

Samsung Bioepis Exhibit No. 1002
Page 1041

It will be appreciated that the methods and compositions of the instant disclosure can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent to the artisan that other embodiments exist and do not depart from the spirit of the disclosure. Thus, the described embodiments are illustrative and should not be construed as restrictive.

Samsung Bioepis Exhibit No. 1002
Page 1042

LIST OF REFERENCES

The publications and other materials used herein to illuminate the background of the disclosure, and in particular, cases to provide additional details respecting the practice, are incorporated herein by reference in their entirety, and for convenience, are referenced by author and date in the text and respectively grouped in the following List of References.

Abbas AK, et al. (2000). Cellular and Molecular Immunology (4th edition), p. 363-383 (W.B. Saunders Company, New York).

Arp et al. (1996). *J. Virol.* 70:7349-7359.

Baldwin et al. (2001). *Immunity* 14:369-376.

Böhmig GA, et al. (2000). *Am. J. Kidney Dis.* 35:667-673.

Brauer et al. (1995). *Transplantation* 59:288-293.

Changelian PS, et al. (2003). *Science* 302:875-878.

Collard et al. (1997). Circulation 96:326-333.

Collins et al. (1999). *J. Am. Soc. Nephrol.* 10:2208-2214.

Fearon (1983). In Intensive Review of Internal Medicine, 2nd Ed. Fanta and Minaker, eds. Brigham and Women's and Beth Israel Hospitals.

Forbes et al. (1978). *Lab. Invest.* 39:463-470.

Frei Y, et al. (1987). *Mol. Cell. Probes* 1:141-149.

Gloor (2005). *Contrib. Nephrol.* 146:11-21.

Glotz et al. (1993). *Transplantation* 56:335-337.

Glotz D, et al. (2002). *Am. J. Transplant.* 2:758-760.

Hakim et al. (1990). *Am. J. Kidney Dis.* 16:423-431.

Halloran PF, et al. (1992). *Transplantation* 53:550-555.

Halloran (2003). *Am. J. Transplant.* 3:639-640.

Haviland DL, et al. (1991). *J. Immunol.* 146:362-368.

Hiesse C, et al. (1992). *Nephrol. Dial. Transplant.* 7:944-951.

Hillmen P, et al. (2004). *New Engl. J. Med.* 350:552-559.

Samsung Bioepis Exhibit No. 1002
Page 1043

Jeannet M, et al. (1970). *New Engl. J. Med.* 282:111-117.

Jones PT, et al. (1986). *Nature* 321:522-525.

Jose et al. (1983). *J. Exp. Med.* 158:2177-2182.

Kirschfink (2001). *Immunol. Rev.* 180:177-189.

Kobayashi et al. (1999). *J. Biol. Chem.* 274:28660-28668.

Kriaa et al. (1995). *Nephrol. Dial. Transplant. 10 Suppl.* 6:108-110.

Kroshus et al. (1995). *Transplantation* 60:1194-1202.

Kupiec-Weglinski (1996). *Ann. Transplant.* 1:34-40.

Kupin et al. (1991). *Transplantation* 51:324-329.

Liszewski (1993). Fundamental Immunology pp. 917-939.

Mauiyyedi et al. (2002). *Curr. Opin. Nephrol. Hypertens.* 11:609-618.

Mehra et al. (2003). *Curr. Opin. Cardiol.* 18:153-158.

Minta JO and Man DP (1977). *J. Immunol.* 119:1597-1602.

Montgomery RA, et al. (2000). *Transplantation* 70:887-895.

Olfert et al. (1993) Guide to the care and use of experimental animals (Vol.1). Ottawa: Association of Universities and Colleges of Canada 1.

Opelz G (1992). *Transplant. Proc.* 24:2342-2355.

OPTN/SRTR Annual Report (2002). Chapter 1 of the Annual Report produced by the Scientific Registry of Transplant Recipients (SRTR) in collaboration with the Organ Procurement and Transplantation Network (OPTN). See http://www.unos.org/data/ar2002/ar02_chapter_one.htm.

Palmer et al. (1989). *Lancet* 1:10-12.

Papadimitriou et al. (1991). *J. Immunol.* 147:212-217.

Park WD et al. (2003). *Am. J. Transplant* 3:952-960.

Persson NH et al. (1995). *Transplant. Proc.* 27:3466.

Platt et al. (1999). *Mol. Immunol.* 36:965-971.

Pratt et al. (1996). *Am J Pathol* 149:2055-2066.

Samsung Bioepis Exhibit No. 1002
Page 1044

Pratt et al. (2000). *Am. J. Pathol.* 157:825-831.

Pruitt et al. (1991). *J. Surg. Res.* 50:350-355.

Regele H, et al. (2001). *Nephrol. Dial. Transplant.* 16:2058-2066.

Rocha et al. (2003). *Transplantation* 75:1490-1495.

Ross et al. (1993). *Transplantation* 55:785-789.

Saadi et al. (1995). *J. Exp. Med.* 182:1807-1814.

Salama et al. (2001). *Am. J. Transplant.* 1:260-269.

Schweitzer et al. (2000). *Transplantation* 70:1531-1536.

Sonnenday et al. (2002). *Transplant. Proc.* 34:1614-1616.

Stepkowski SM (2000). *Exp. Rev. Mol. Med.* 21 June, http://www-ermm.cbcu.cam.ac.uk /00001769h.htm.

Taube DH, et al. (1984). *Lancet* 1:824-828.

Tyan et al. (1994). *Transplantation* 57:553-562.

Vakeva et al. (1998). *Circulation* 97:2259-2267.

Vogt W, et al. (1989). *Molec. Immunol.* 26:1133-1142.

Wang et al. (1995). *Proc. Natl. Acad. Sci. U. S. A.* 92:8955-8959.

Wang et al. (1996). *Proc. Natl. Acad. Sci. U. S. A.* 93:8563-8568.

Wang et al. (1999). *Transplantation* 68:1643-1651.

Wang H, et al. (2003). *J. Immunol.* 171:3823-3836.

Warren et al. (2004). *Am. J. Transplant.* 4:561-568.

Wetsel RA and Kolb WP (1982). *J. Immunol.* 128:2209-2216.

Wurzner R, et al. (1991). *Complement Inflamm.* 8:328-340.

Samsung Bioepis Exhibit No. 1002
Page 1045

Yamamoto KI and Gewurz G (1978). *J. Immunol.* 120:2008-2015.

PCT Patent Application Publication WO 92/10205

PCT Patent Application Publication WO 00/27421

PCT Patent Application Publication WO 01/37860

U.S. Patent Application Publication US 2003/0129187

U.S. Patent Application Publication US 2003/0180301

U.S. Patent 5,133,916

U.S. Patent 5,573,940

U.S. Patent 5,660,825

U.S. Patent 6,057,131

U.S. Patent 6,100,443

U.S. Patent 6,192,891

U.S. Patent 6,280,957

U.S. Patent 6,302,855

U.S. Patent 6,355,245

U.S. Patent 6,534,058

U.S. Patent 6,821,515

The contents of all references cited herein are incorporated by reference in their entirety.

Samsung Bioepis Exhibit No. 1002
Page 1046

CLAIMS

1. A method to prolong survival of an MHC mismatched allograft in a recipient mammal, said method comprising administering to said mammal a) a drug which inhibits complement activity and b) at least one immunosuppressive drug, wherein said drug which inhibits complement activity is administered chronically.

2. The method of claim 1 wherein said mammal is a human.

3. The method of claim 2 wherein said MHC mismatched allograft is an HLA mismatched allograft.

4. The method of claim 1 wherein said drug which inhibits complement activity inhibits the formation of terminal complement or C5a.

5. The method of claim 4 wherein said drug which inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment.

6. The method of claim 5 wherein said whole antibody or antibody fragment is a human, humanized, chimerized or deimmunized antibody or antibody fragment.

7. The method of claim 5 wherein said whole antibody or antibody fragment inhibits cleavage of complement C5.

8. The method of claim 5 wherein said antibody fragment is selected from the group consisting of an Fab, an $F(ab')_2$, an Fv, a domain antibody, and a single-chain antibody.

9. The method of claim 5 wherein said antibody fragment is pexelizumab.

10. The method of claim 5 wherein said whole antibody is eculizumab.

11. The method claim 10 wherein said eculizumab is administered once every 2 weeks.

12. The method of claim 1 wherein said inhibitor of complement activity is selected from the group consisting of a i) soluble complement receptor, ii) CD59, iii) CD55, iv) CD46, and v) an antibody to C5, C6, C7, C8, or C9.

13. The method of claim 1 wherein said immunosuppressive drug inhibits T-cell activity or B-cell activity.

Samsung Bioepis Exhibit No. 1002
Page 1047

14. The method of claim 1 wherein said immunosuppressive drug inhibits T-cell activity and B-cell activity.

15. The method of claim 1 wherein said immunosuppressive drug is selected from the group consisting of cyclosporin A, tacrolimus, sirolimus, OKT3, a corticosteroid, daclizumab, basiliximab, azathioprene, mycophenolate mofetil, methotrexate, 6-mercaptopurine, anti-T cell antibodies, cyclophosphamide, leflunamide, brequinar, ATG, ALG, 15-deoxyspergualin, and bredinin.

16. The method of claim 1 wherein more than one immunosuppressive drug is administered.

17. The method of claim 1 wherein said method comprises administering i) a drug which inhibits complement activity and ii) cyclosporin A.

18. The method of claim 17 wherein said drug which inhibits complement activity is an antibody which inhibits cleavage of complement C5.

19. The method of claim 1 wherein said allograft is selected from the group consisting of i) heart, ii) kidney, iii) lung, iv) pancreas, v) liver, vi) vascular tissue, vii) eye, viii) cornea, ix) lens, x) skin, xi) bone marrow, xii) muscle, xiii) connective tissue, xiv) gastrointestinal tissue, xv) nervous tissue, xvi) bone, xvii) stem cells, xviii) islets, xix) cartilage, xx) hepatocytes, and xxi) hematopoietic cells.

20. The method of claim 1 wherein said allograft survives for a time at least 20% longer than would occur if said method were to be performed without said drug which inhibits complement activity.

21. The method of claim 1 wherein said allograft survives for a time at least 40% longer than would occur if said method were to be performed without said drug which inhibits complement activity.

22. The method of claim 1 wherein said allograft survives for the remaining life-time of said mammal.

23. The method of claim 2 wherein said allograft survives for at least six months.

24. The method of claim 2 wherein said allograft survives for at least one year.

25. The method of claim 2 wherein said allograft survives for at least five years.

Samsung Bioepis Exhibit No. 1002
Page 1048

26. The method of claim 2 wherein said allograft survives for the remaining life-time of said human.

27. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 14 days.

28. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 28 days.

29. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 3 months.

30. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 6 months.

31. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 1 year.

32. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 5 years.

33. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for the remaining life-time of said mammal.

34. The method of claim 1 wherein at least one immunosuppressive drug is administered chronically for the remaining life-time of said mammal.

35. The method of claim 34 wherein at least cyclosporin A is administered chronically for the remaining life-time of said mammal.

36. A method to prolong survival of an allograft in a recipient mammal presensitized to said allograft, said method comprising administering to said mammal a) a drug which inhibits complement activity and b) at least one immunosuppressive drug, wherein said drug which inhibits complement activity is administered chronically.

37. The method of claim 36 wherein said mammal is a human.

38. The method of claim 36 wherein said drug which inhibits complement activity inhibits the formation of terminal complement or C5a.

Samsung Bioepis Exhibit No. 1002
Page 1049

39. The method of claim 38 wherein said drug that inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment.

40. The method of claim 39 wherein said whole antibody or antibody fragment is a human, humanized, chimerized or deimmunized antibody or antibody fragment.

41. The method of claim 39 wherein said whole antibody or antibody fragment inhibits cleavage of complement C5.

42. The method of claim 39 wherein said antibody fragment is selected from the group consisting of an Fab, an $F(ab')_2$, an Fv, a domain antibody, and a single-chain antibody.

43. The method of claim 39 wherein said antibody fragment is pexelizumab.

44. The method of claim 39 wherein said whole antibody is eculizumab.

45. The method claim 44 wherein said eculizumab is administered once every 2 weeks.

46. The method of claim 36 wherein said inhibitor of complement activity is selected from the group consisting of a i) soluble complement receptor, ii) CD59, iii) CD55, iv) CD46, and v) an antibody to C5, C6, C7, C8, or C9.

47. The method of claim 36 wherein said immunosuppressive drug inhibits T-cell activity or B-cell activity.

48. The method of claim 36 wherein said immunosuppressive drug inhibits T-cell activity and B-cell activity.

49. The method of claim 36 wherein said immunosuppressive drug is selected from the group consisting of cyclosporin A, tacrolimus, sirolimus, OKT3, a corticosteroid, daclizumab, basiliximab, azathioprene, mycophenolate mofetil, methotrexate, 6-mercaptopurine, anti-T cell antibodies, cyclophosphamide, leflunamide, brequinar, ATG, ALG, 15-deoxyspergualin, and bredinin.

50. The method of claim 36 wherein more than one immunosuppressive drug is administered.

51. The method of claim 36 wherein said method comprises administering i) a drug which inhibits complement activity and ii) cyclosporin A.

Samsung Bioepis Exhibit No. 1002
Page 1050

52. The method of claim 51 wherein said method comprises administering i) a drug which inhibits complement activity, ii) cyclosporin A, and iii) cyclophosphamide.

53. The method of claim 51 wherein said drug which inhibits complement activity is an antibody which inhibits cleavage of complement C5.

54. The method of claim 36 wherein said allograft is selected from the group consisting of i) heart, ii) kidney, iii) lung, iv) pancreas, v) liver, vi) vascular tissue, vii) eye, viii) cornea, ix) lens, x) skin, xi) bone marrow, xii) muscle, xiii) connective tissue, xiv) gastrointestinal tissue, xv) nervous tissue, xvi) bone, xvii) stem cells, xviii) islets, xix) cartilage, xx) hepatocytes, and xxi) hematopoietic cells.

55. The method of claim 36 wherein said allograft survives for a time at least 20% longer than would occur if said method were to be performed without said drug which inhibits complement activity.

56. The method of claim 36 wherein said allograft survives for a time at least 40% longer than would occur if said method were to be performed without said drug which inhibits complement activity.

57. The method of claim 36 wherein said allograft survives for the remaining life-time of said mammal.

58. The method of claim 37 wherein said allograft survives for at least six months.

59. The method of claim 37 wherein said allograft survives for at least one year.

60. The method of claim 37 wherein said allograft survives for at least five years.

61. The method of claim 37 wherein said allograft survives for the remaining life-time of said human.

62. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 14 days.

63. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 28 days.

64. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 3 months.

Samsung Bioepis Exhibit No. 1002
Page 1051

65. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 6 months.

66. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 1 year.

67. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 5 years.

68. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for the remaining life-time of said mammal.

69. The method of claim 36 wherein at least one immunosuppressive drug is administered chronically for the remaining life-time of said mammal.

70. The method of claim 69 wherein at least cyclosporin A is administered chronically for the remaining life-time of said mammal.

71. A method to prolong survival of an allograft in a recipient mammal which is an ABO mismatch to said allograft, said method comprising administering to said mammal a) a drug which inhibits complement activity and b) at least one immunosuppressive drug, wherein said drug which inhibits complement activity is administered chronically.

72. The method of claim 71 wherein said mammal is a human.

73. The method of claim 71 wherein said drug which inhibits complement activity inhibits the formation of terminal complement or C5a.

74. The method of claim 73 wherein said drug which inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment.

75. The method of claim 74 wherein said whole antibody or antibody fragment is a human, humanized, chimerized or deimmunized antibody or antibody fragment.

76. The method of claim 74 wherein said whole antibody or antibody fragment inhibits cleavage of complement C5.

77. The method of claim 74 wherein said antibody fragment is selected from the group consisting of an Fab, an $F(ab')_2$, an Fv, a domain antibody, and a single-chain antibody.

Samsung Bioepis Exhibit No. 1002
Page 1052

78. The method of claim 74 wherein said antibody fragment is pexelizumab.

79. The method of claim 74 wherein said whole antibody is eculizumab.

80. The method claim 79 wherein said eculizumab is administered once every 2 weeks.

81. The method of claim 71 wherein said inhibitor of complement activity is selected from the group consisting of a i) soluble complement receptor, ii) CD59, iii) CD55, iv) CD46, and v) an antibody to C5, C6, C7, C8, or C9.

82. The method of claim 71 wherein said immunosuppressive drug inhibits T-cell activity or B-cell activity.

83. The method of claim 71 wherein said immunosuppressive drug inhibits T-cell activity and B-cell activity.

84. The method of claim 71 wherein said immunosuppressive drug is selected from the group consisting of cyclosporin A, tacrolimus, sirolimus, OKT3, a corticosteroid, daclizumab, basiliximab, azathioprene, mycophenolate mofetil, methotrexate, 6-mercaptopurine, anti-T cell antibodies, cyclophosphamide, leflunamide, brequinar, ATG, ALG, 15-deoxyspergualin, and bredinin.

85. The method of claim 71 wherein more than one immunosuppressive drug is administered.

86. The method of claim 71 wherein said method comprises administering i) a drug which inhibits complement activity and ii) cyclosporin A.

87. The method of claim 86 wherein said method comprises administering i) a drug which inhibits complement activity, ii) cyclosporin A, and iii) cyclophosphamide.

88. The method of claim 86 wherein said drug that inhibits complement activity is an antibody that inhibits cleavage of complement C5.

89. The method of claim 71 wherein said allograft is selected from the group consisting of i) heart, ii) kidney, iii) lung, iv) pancreas, v) liver, vi) vascular tissue, vii) eye, viii) cornea, ix) lens, x) skin, xi) bone marrow, xii) muscle, xiii) connective tissue, xiv) gastrointestinal tissue, xv) nervous tissue, xvi) bone, xvii) stem cells, xviii) islets, xix) cartilage, xx) hepatocytes, and xxi) hematopoietic cells.

Samsung Bioepis Exhibit No. 1002
Page 1053

90. The method of claim 71 wherein said allograft survives for a time at least 20% longer than would occur if said method were to be performed without said drug that inhibits complement activity.

91. The method of claim 71 wherein said allograft survives for a time at least 40% longer than would occur if said method were to be performed without said drug that inhibits complement activity.

92. The method of claim 71 wherein said allograft survives for the remaining life-time of said mammal.

93. The method of claim 72 wherein said allograft survives for at least six months.

94. The method of claim 72 wherein said allograft survives for at least one year.

95. The method of claim 72 wherein said allograft survives for at least five years.

96. The method of claim 72 wherein said allograft survives for the remaining life-time of said human.

97. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 14 days.

98. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 28 days.

99. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 3 months.

100. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 6 months.

101. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 1 year.

102. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 5 years.

103. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for the remaining life-time of said mammal.

Samsung Bioepis Exhibit No. 1002
Page 1054

104. The method of claim 71 wherein at least one immunosuppressive drug is administered chronically for the remaining life-time of said mammal.

105. The method of claim 104 wherein at least cyclosporin A is administered chronically for the remaining life-time of said mammal.

106. Use of a drug that inhibits complement activity and an immunosuppressive drug in the manufacture of a medicament or medicament package for prolong survival of an allograft in a mammal.

107. The use of claim 106, wherein more than one immunosuppressive drug is included in said medicament or medicament package.

108. The use of claim 106, wherein said drug that inhibits complement activity and said immunosuppressive drug are in a formulation suitable for concurrent administration to said mammal.

109. The use of claim 106, wherein said drug that inhibits complement activity and said immunosuppressive drug are in a formulation or formulations suitable for sequential administration to said mammal.

110. The use of claim 106, wherein said drug that inhibits complement activity is in a formulation suitable for chronic administration to said mammal.

111. The use of claim 106, wherein said immunosuppressive drug is in a formulation suitable for chronic administration to said mammal.

112. A method to prolong survival of allograft in a recipient mammal, comprising preparing an allograft from a first allograft accommodated by a first recipient mammal, wherein said first recipient mammal, after receiving said first allograft, has been treated with a drug that inhibits complement activity and at least one immunosuppressive agent.

113. An allograft that has prolonged survival in a recipient mammal, wherein said allograft is prepared from a first recipient mammal and was transplanted to the first recipient mammal that has been treated with a drug that inhibits complement activity and at least one immunosuppressive agent.

Samsung Bioepis Exhibit No. 1002
Page 1055

114. A pharmaceutical package comprising a drug that inhibits complement activity and at least one immunosuppressive agent, wherein said drug and said agent are formulated for chronic administration.

115. The pharmaceutical package of claim 114, wherein said drug is an antibody in a lyophilized formulation comprising the antibody and a lyoprotectant.

116. The pharmaceutical package of claim 114, wherein said agent is in a lyophilized formulation comprising the agent and a lyoprotectant.

117. The pharmaceutical package of claim 114, wherein said drug and said agent are in the same lyophilized formulation comprising said drug, said agent, and a lyoprotectant.

118. The pharmaceutical package of claim 114, wherein said drug is in an injection system comprising a syringe that comprises a cartridge, wherein said cartridge contains said drug in a formulation suitable for injection.

119. The pharmaceutical package of claim 114, wherein said agent is in an injection system comprising a syringe that comprises a cartridge, wherein said cartridge contains said agent in a formulation suitable for injection.

120. The pharmaceutical package of claim 114, wherein said drug and said agent are in an injection system comprising a syringe that comprises a cartridge, wherein said cartridge contains said drug and said agent in a formulation suitable for injection.

121. The pharmaceutical package of claim 114, wherein said drug is present in unit dosage form.

122. The pharmaceutical package of claim 114, wherein said agent is present in unit dosage form.

123. A lyophilized antibody formulation comprising an antibody that inhibits complement activity and a lyoprotectant, wherein said antibody formulation is suitable for chronic administration.

124. The lyophilized antibody formulation of claim 123, wherein the antibody inhibits the formation of terminal complement or C5a.

Samsung Bioepis Exhibit No. 1002
Page 1056

125. The lyophilized antibody formulation of claim 123, wherein the antibody is a whole antibody or an antibody fragment.

126. The lyophilized antibody formulation of claim 125, wherein the antibody wherein said whole antibody or antibody fragment is a human, humanized, chimerized or deimmunized antibody or antibody fragment.

127. The lyophilized antibody formulation of claim 125, wherein said whole antibody or antibody fragment inhibits cleavage of complement C5.

128. The lyophilized antibody formulation of claim 125, wherein said antibody fragment is selected from the group consisting of an Fab, an $F(ab')_2$, an Fv, a domain antibody, and a single-chain antibody.

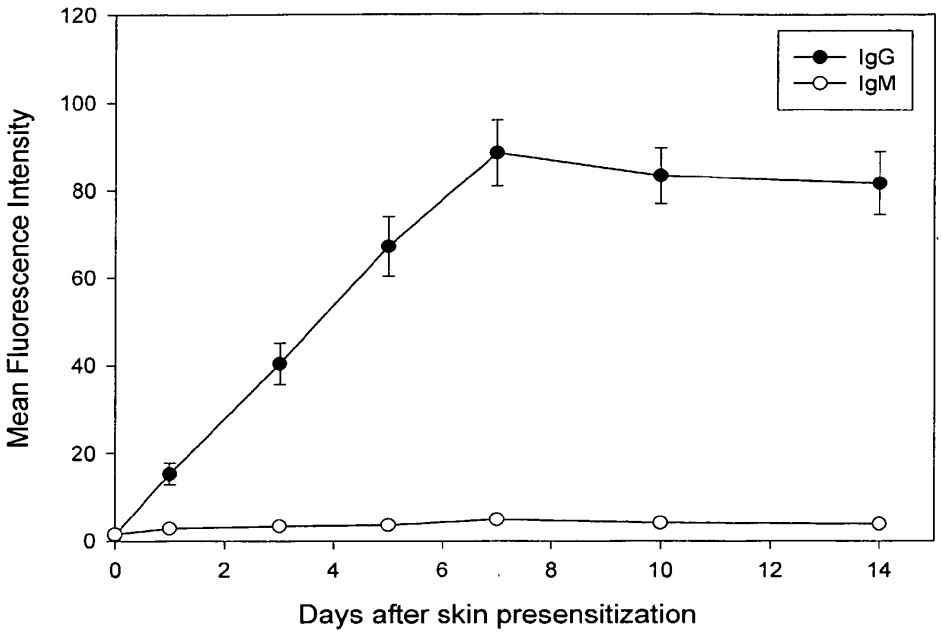
129. The lyophilized antibody formulation of claim 125, wherein said antibody fragment is pexelizumab.

130. The lyophilized antibody formulation of claim 125, wherein said whole antibody is eculizumab.

131. An injection system comprising a syringe, wherein said syringe comprises a cartridge containing an antibody that inhibits complement activity and is formulated suitable for injection.

Samsung Bioepis Exhibit No. 1002
Page 1057

A



B

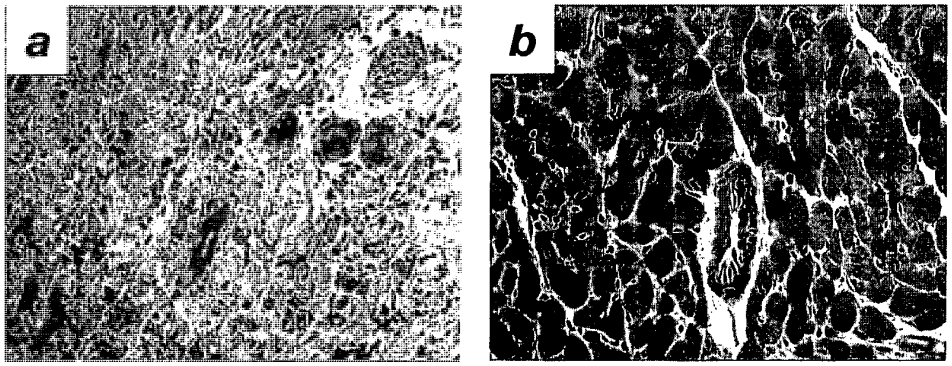


Figure 1A, B

Samsung Bioepis Exhibit No. 1002
Page 1058

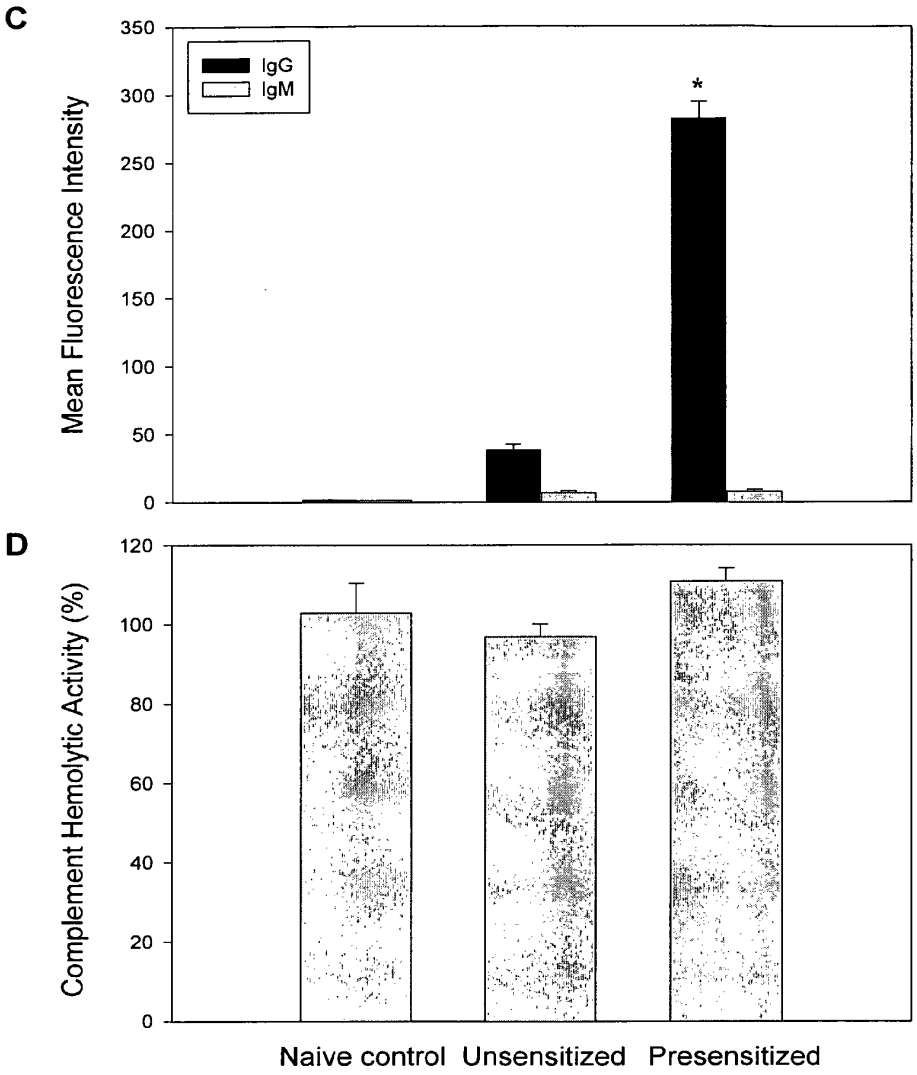


Figure 1C, D

Samsung Bioepis Exhibit No. 1002
Page 1059

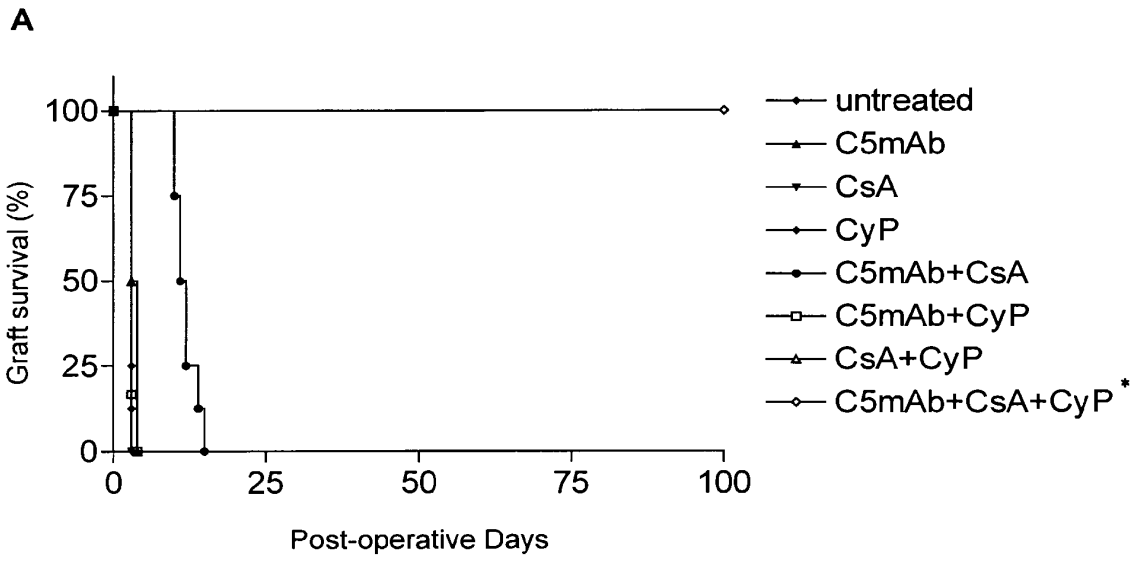

A
Graft survival (%)
100
75
50
25
0
0
25
50
75
100
Post-operative Days
untreated
C5mAb
CsA
CyP
C5mAb+CsA
C5mAb+CyP
CsA+CyP
C5mAb+CsA+CyP *

Figure 2A

B

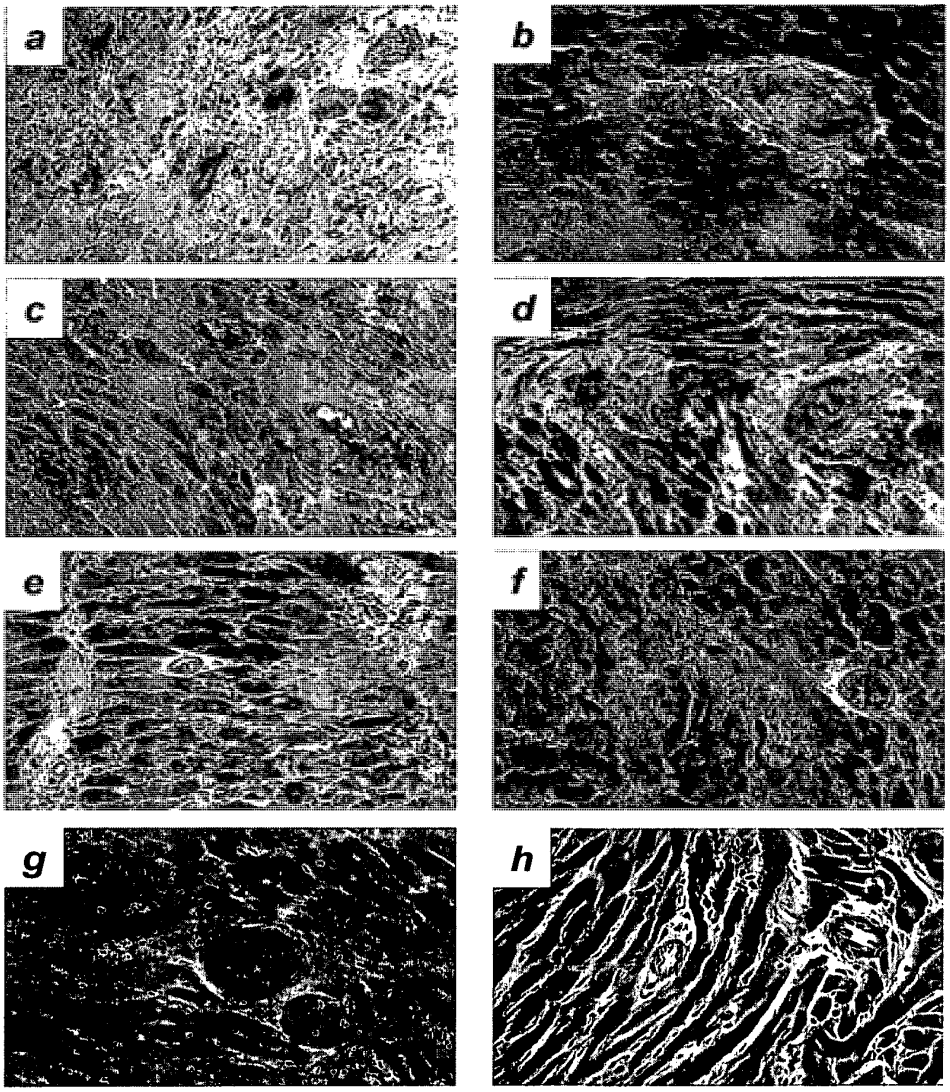


Figure 2B

Samsung Bioepis Exhibit No. 1002
Page 1061

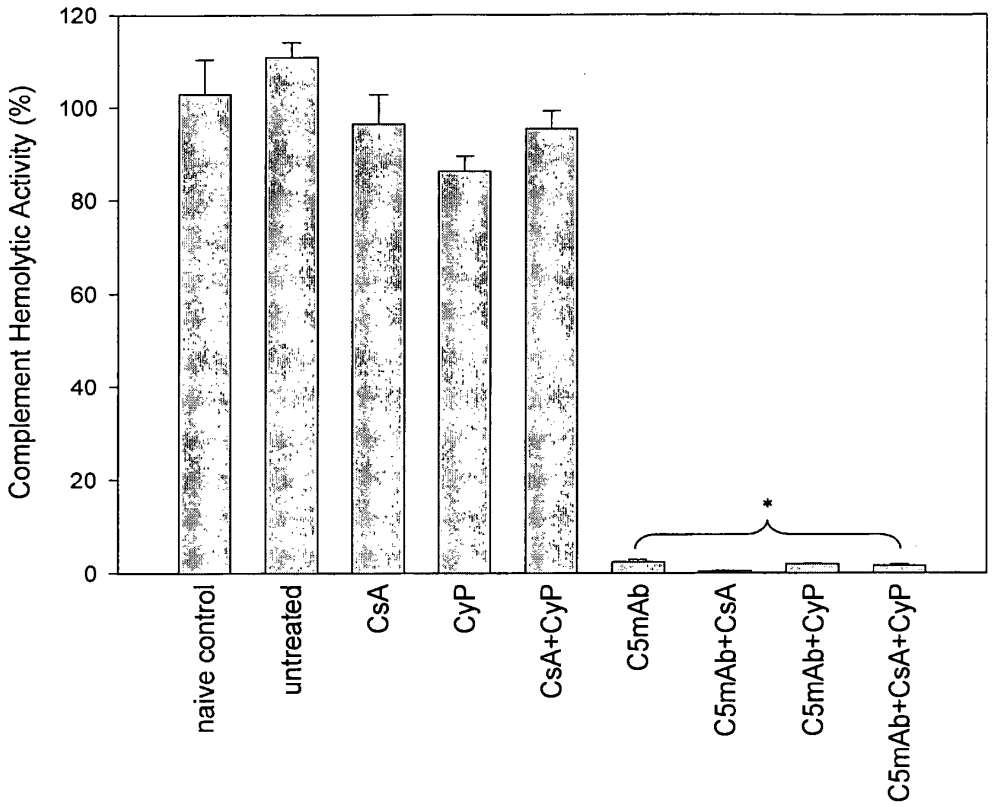


Figure 3

Samsung Bioepis Exhibit No. 1002
Page 1062

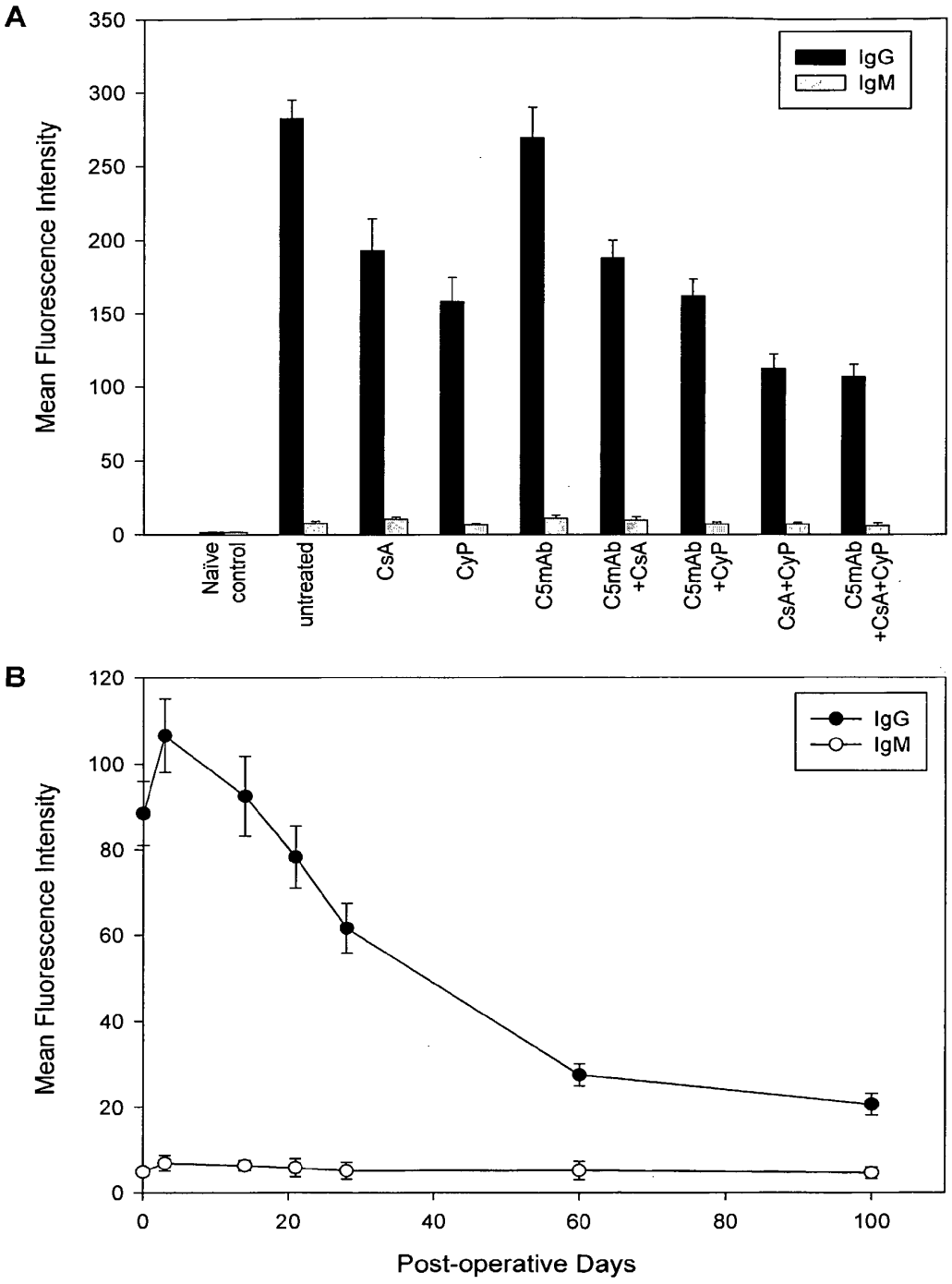
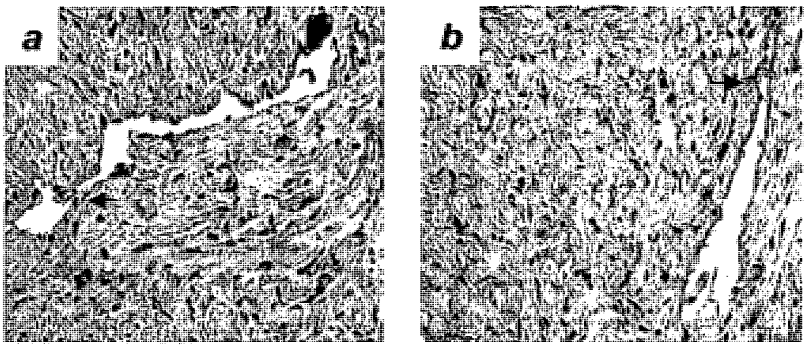


Figure 4A, B

Samsung Bioepis Exhibit No. 1002
Page 1063

C



D

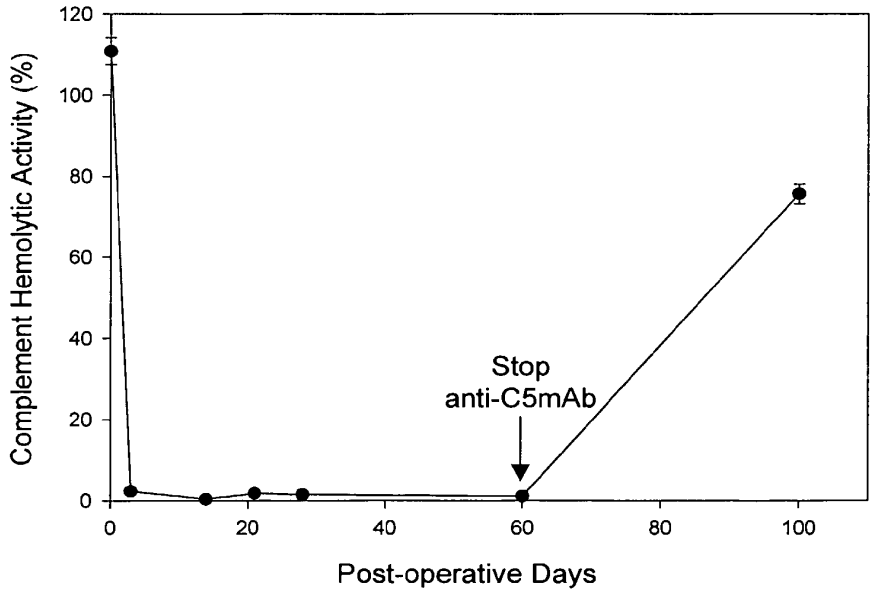


Figure 4C, D

Samsung Bioepis Exhibit No. 1002
Page 1064

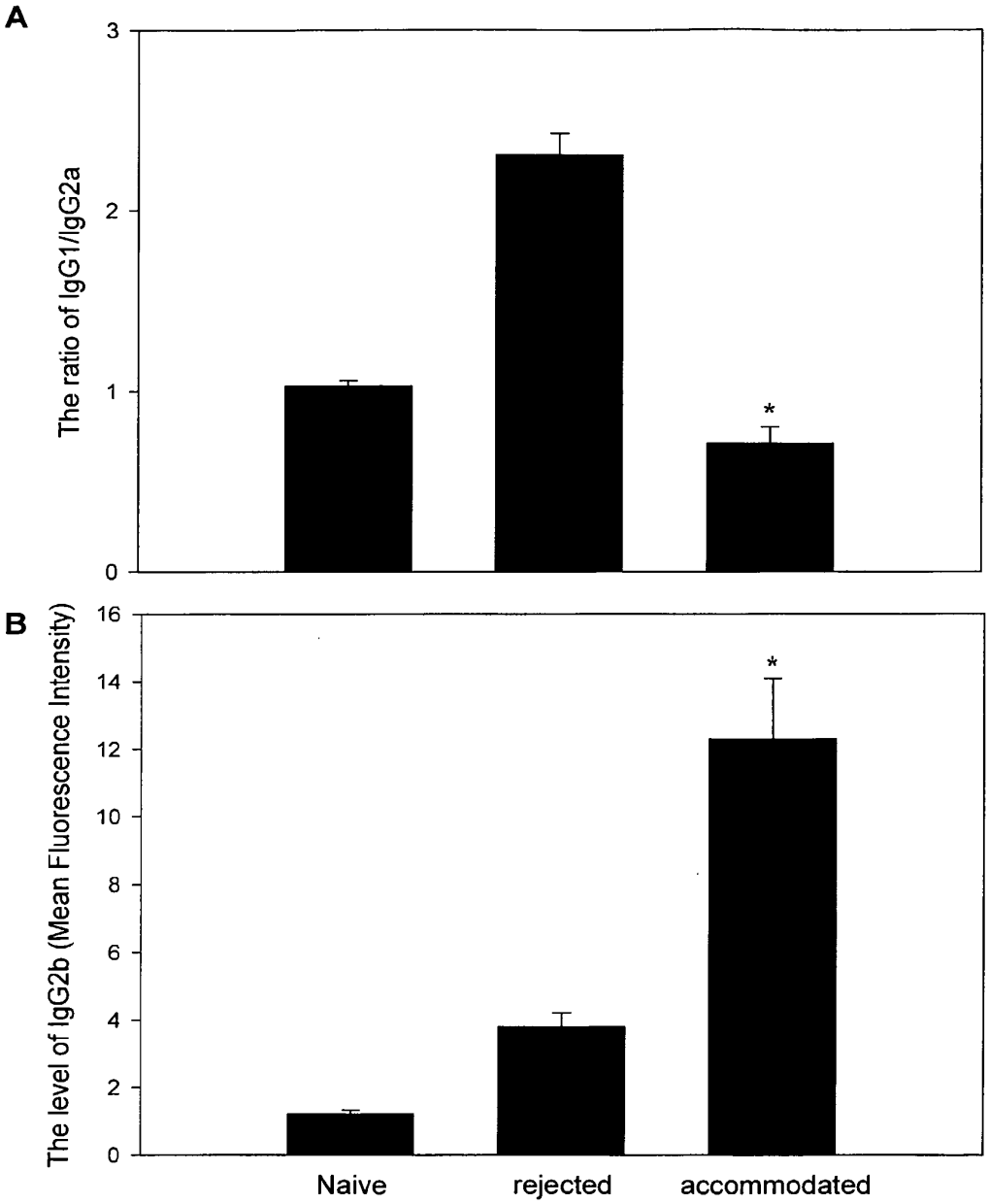


Figure 5A, B

Samsung Bioepis Exhibit No. 1002
Page 1065

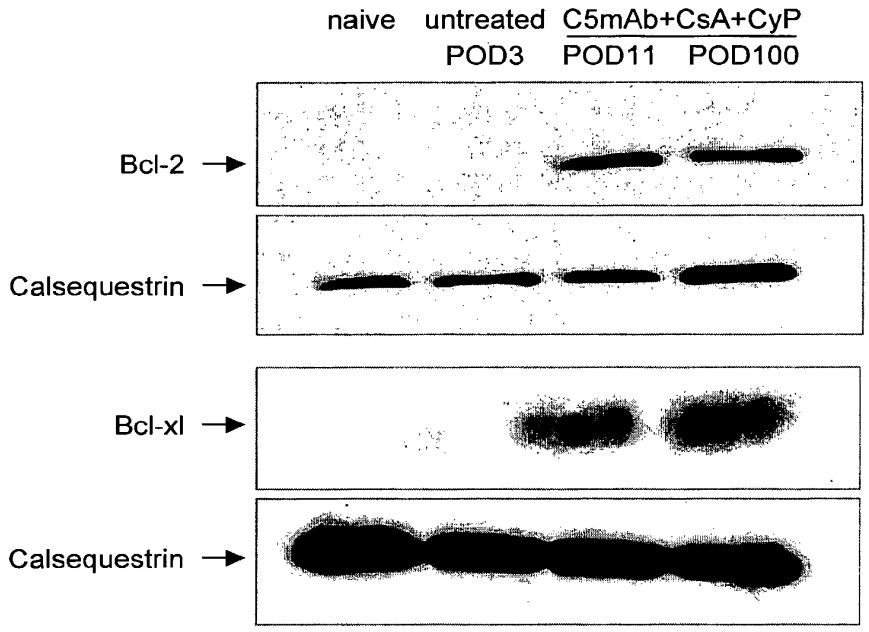


Figure 6

Samsung Bioepis Exhibit No. 1002
Page 1066

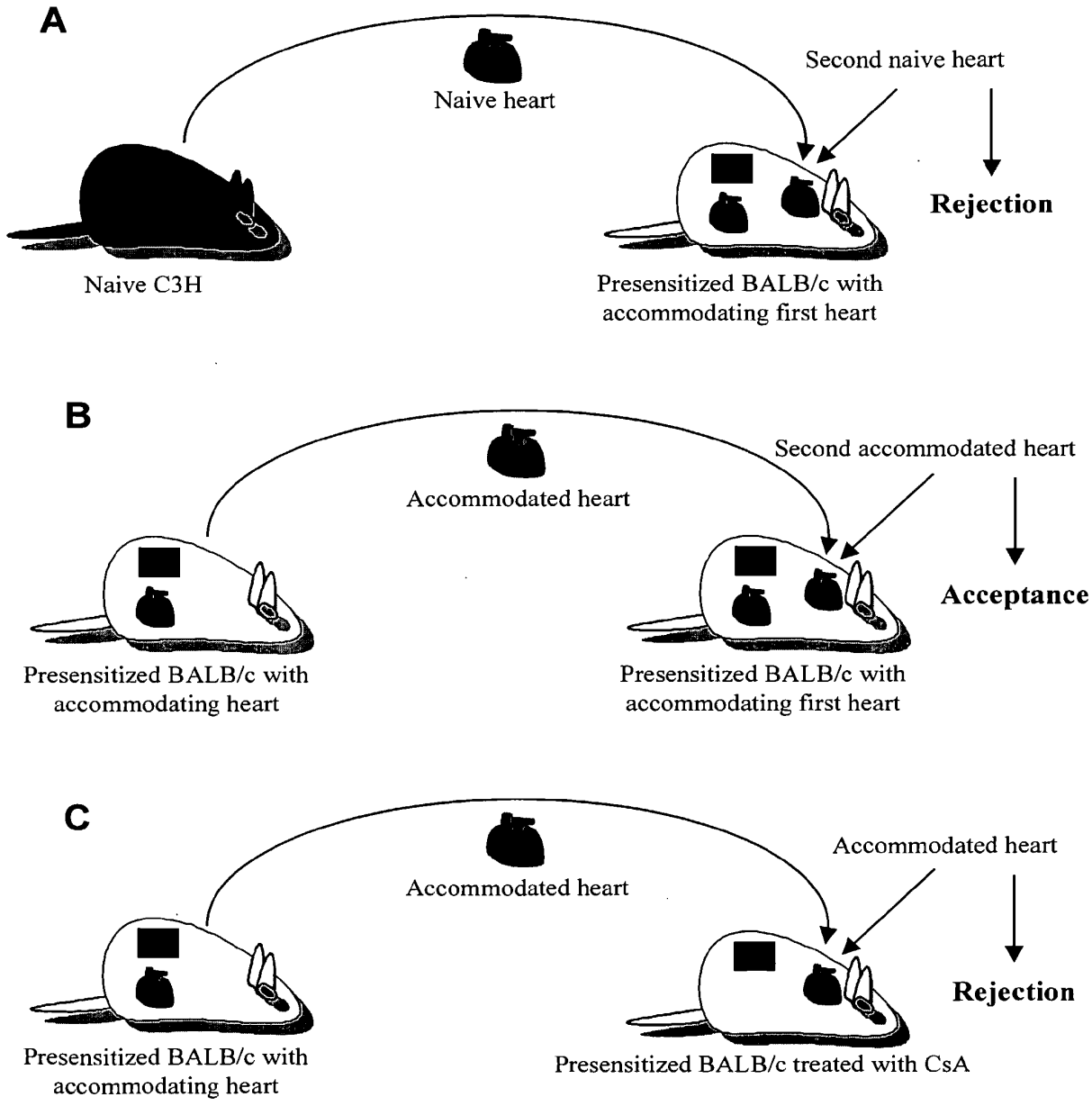


Figure 7

Samsung Bioepis Exhibit No. 1002
Page 1067

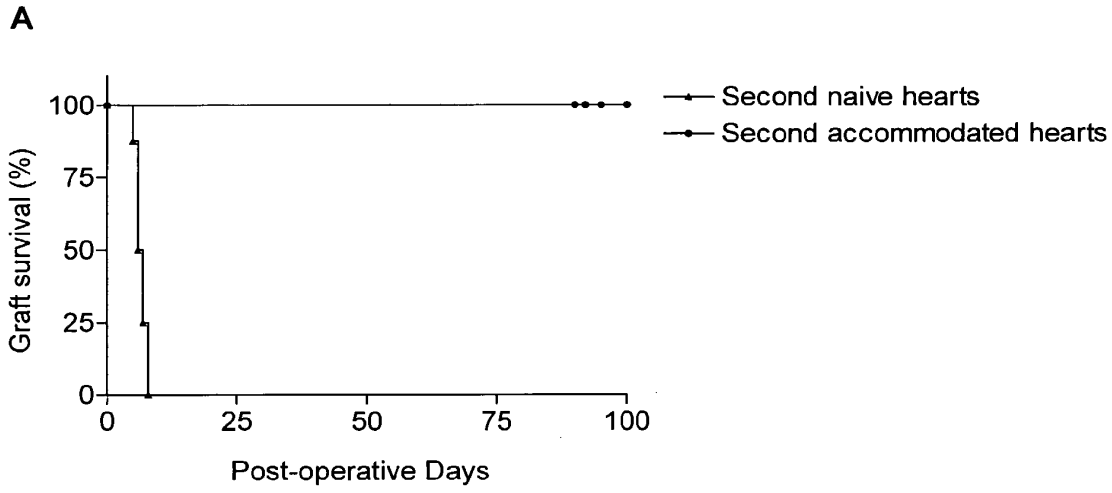


Figure 8

Samsung Bioepis Exhibit No. 1002
Page 1068

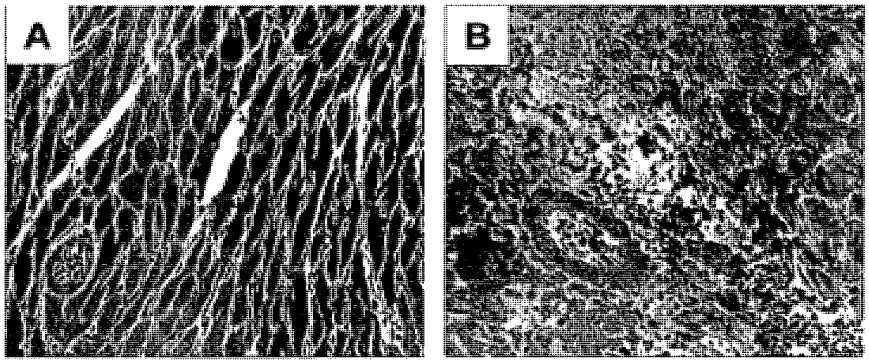


Figure 9

Samsung Bioepis Exhibit No. 1002
Page 1069

T1897/14-3.3.04
(05 71 2607.0 / 1720571)
Patentee: Alexion Pharmaceuticals, Inc.
Opposition by: Pfizer Inc.
Our Ref.: M2367 OPP(EP)

DRUG NAME: Alemtuzumab

SYNONYM(S): Campath, Ldp 103, Mabcambath, MabCampath-1H

COMMON TRADE NAME(S): MABCAMPATH®

CLASSIFICATION: monoclonal antibody

Special pediatric considerations are noted when applicable, otherwise adult provisions apply.

MECHANISM OF ACTION:

Alemtuzumab is a recombinant human IgG-derived monoclonal antibody that binds to antigen CD52 which is found on the surface of B and T lymphocytes, most monocytes, macrophages and NK cells, and certain granulocytes, but not hematopoietic stem cells.[1] Lysis of CD52-positive cells occurs via complement activation, antibody-dependent cytotoxicity, and apoptosis.[2]

PHARMACOKINETICS:

Table refers to intravenous (IV) dosing. Subcutaneous (SC) administration of alemtuzumab achieves concentrations similar to those with intravenous alemtuzumab, although with a slightly higher cumulative dose.[3,4]

Distribution	cross blood brain barrier?	no information found
	volume of distribution	mean 0.18 L/kg (range 0.1-0.4 L/kg)
	plasma protein binding	no information found
Metabolism	no information found	
	active metabolite(s)	no information found
	inactive metabolite(s)	no information found
Excretion	no information found	
	terminal half life	mean 11 h (range 2-32 h) after the first dose; 6 d (range 1-14 d) after 12 weeks
	clearance	wide variability, affected by tumour burden[5]; clearance decreases with repeated administration due to decreased receptor mediated clearance (loss of CD52 receptors in the periphery)
Elderly	comparable AUC in patients older than 65 years of age	

Adapted from standard reference[1] unless specified otherwise.

USES:

Primary uses:
*B-cell chronic lymphocytic leukemia[1,6]
T-cell prolymphocytic leukemia[6-8]

*Health Canada approved indication

Other uses:
Cutaneous T-cell lymphoma[7]
Peripheral T-cell lymphoma[7]

Samsung Bioepis Exhibit No. 1002
Page 1070

SPECIAL PRECAUTIONS:

Contraindicated in patients who have a history of hypersensitivity reaction to alemtuzumab, active infections, underlying immunodeficiency (e.g., seropositive for HIV), or other active malignancies.[1]

Caution:

- in patients with previous hypersensitivity or anaphylactic reactions to other monoclonal antibodies[9]
- gradual dose escalation and appropriate pre-medication is required; for more information, see paragraph following the **Side Effects** table[1]
- single doses of alemtuzumab greater than 30 mg or cumulative doses greater than 90 mg per week should not be administered as they are associated with a higher incidence of pancytopenia[1]
- transfusion-related graft-versus-host-disease can occur in patients with severe lymphopenia and can be life threatening; patients receiving alemtuzumab should receive irradiated blood products, effectively eliminating this risk[1]
- *Pneumocystis* pneumonia (PCP) and herpes virus prophylaxis are recommended upon initiation of therapy[1,6,10,11]
- use alemtuzumab with caution in patients with recent exposure to the chicken pox virus[9]

Hepatitis B (HBV) reactivation: All lymphoma patients should be tested for both HBsAg and HBcAb. If either test is positive, such patients should be treated with lamivudine 100 mg/day orally, for the entire duration of chemotherapy and for six months afterwards.[5,10] Such patients should also be monitored with frequent liver function tests and HBV DNA at least every two months. If the HBV DNA level rises during this monitoring, management should be reviewed with a hepatologist and consideration given to halting chemotherapy.[6]

Cytomegalovirus reactivation: for more information, see paragraph following the **Side Effects** table

Carcinogenicity: no information found[1]

Mutagenicity: no information found[1]

Fertility: Alemtuzumab has been shown to bind to mature sperm.[1] No long term studies have been performed on men or women to determine the effect on infertility. Patients of reproductive potential should use effective contraceptive methods during treatment and for a minimum of 6 months following alemtuzumab therapy.[1]

Pregnancy: FDA Pregnancy Category C.[7] Either studies in animals have revealed adverse effects on the fetus (teratogenic or embryocidal or other), and there are no controlled studies in women, or studies in women and animals are not available. Alemtuzumab should be given only if the potential benefit justifies the potential risk to the fetus. Human IgG is known to cross the placental barrier, therefore alemtuzumab may also cross the placenta and cause fetal B and T lymphocyte depletion.[1]

Breastfeeding should be discontinued during treatment and for at least 3 months following the last dose of alemtuzumab as human IgG is excreted in breast milk.[1]

SIDE EFFECTS:

The table includes adverse events that presented during drug treatment but may not necessarily have a causal relationship with the drug. Because clinical trials are conducted under very specific conditions, the adverse event rates observed may not reflect the rates observed in clinical practice. Adverse events are generally included if they were reported in more than 1% of patients in the product monograph or pivotal trials, and/or determined to be clinically important.[12] When placebo-controlled trials are available, adverse events are included if the incidence is $\geq$ 5% higher in the treatment group.

Samsung Bioepis Exhibit No. 1002
Page 1071

Table refers to IV data unless specified. SC hematological and infectious complications are similar to IV.

ORGAN SITE	SIDE EFFECT
Clinically important side effects are in ***bold, italics***	
allergy/immunology	anaphylactic reaction (<1%)[13] first dose reactions, infusion-related reactions with SC use, injection site reactions and fever
	angioedema (<1%)[13]
	antibody development (2%)
	autoimmune diseases; Graves' disease, Guillain-Barre syndrome, hypothyroidism, Goodpasture's syndrome, autoimmune thyroiditis[14]
	mouth edema (1%, severe 1%)
blood/bone marrow/ febrile neutropenia	anemia (77-80%, severe 38%)[13]; autoimmune hemolytic anemia (1%)[13]
	atypical lymphocytes (1%, severe 1%)
	immunosuppression,[1,15] lymphopenia (100%)[15]; onset in 2-4 weeks, may persist for over one year[10]
	neutropenia (85%, severe 64%)[13]; during the first weeks of therapy,[15] nadir 4-6 weeks,[15] median duration 21 days[13]; febrile neutropenia (5-10%, severe 1%)[13]
	pancytopenia (5%, severe 3%),
	thrombocytopenia (72%, severe 50%) nadir 2-4 weeks,[15] median duration 21 d (range 2-165 d); autoimmune idiopathic thrombocytopenia (2%)
cardiovascular (arrhythmia)	arrhythmias (<1%)[13]
	tachycardia (5-11%, severe 1%)[13]
cardiovascular (general)	cardiac ischemia, infarction (<1%)[13,16,17]
	hypotension (30-32%, severe 3%)[13]; with SC use (0%)[3]
	hypertension (9-11%, severe 1%)[13]
coagulation	disseminated intravascular coagulation (<1%)[13,18]
	deep vein thrombosis, pulmonary embolism (<1%)[13]
	thrombocytopenic purpura (2%, severe 0%)
constitutional symptoms	asthenia (9%, severe 0%), malaise (5%, severe 0%), fatigue (27-34%, severe 4%)[13]; with SC use (5%, severe 2%)[3]
	fever (82-85%, severe 14%)[13]; usually occurs 5-6 hours after the start of the infusion[19] with SC use (70%, severe 2%)[3]
	insomnia (1-10%, severe 0%)[13]
	rigors (85%, severe 15%); with SC use (17%, severe 2%)[3]
	sweating (15%, severe 1%)
	temperature change sensation (5%, severe 0%)
	weight loss (5%, severe 0%)
dermatology/skin	*extravasation hazard: none*[20]
	bullous eruptions (1%, severe 1%)
	erythematous rash (4%, severe 1%)
	flushing (4%, severe 0%)

Samsung Bioepis Exhibit No. 1002
Page 1072

ORGAN SITE	SIDE EFFECT
Clinically important side effects are in ***bold, italics***	
	injection site reaction (1%, severe 0%) with SC use (90%, severe 2%)[3]; onset approximately 24 h after the first dose, often persists until the fourth administration[21]
	pruritus/urticaria (21-30%, severe 1-5%)[13]; with SC use (0%)[3]
	rash (29-40%, severe 3%)[13]; with SC use (0%)[3]
endocrine	ADH secretion abnormality (<1%)[22]
	hyperthyroidism (<1%)[1]
gastrointestinal	*emetogenic potential: rare*[23]
	anorexia (13-20%, severe 1%)[13]
	ascites (<1%)[13]
	colitis, enteritis (<1%)
	constipation (2%, severe 0%)
	dehydration (2%, severe 2%)
	diarrhea (13-22%, severe 1%)[13]; with SC use (0%)[3]
	dyspepsia (4%, severe 0%); ulcer (<1%)[13]
	mucositis (2%, severe 0%)
	nausea (49-54%, severe 2%)[13]; late nausea is rare[15] with SC use (0%)[3]
	stomatitis (5%, severe 1%); ulcerative stomatitis (3%, severe 0%)
	taste loss (2%, severe 1%)
	vomiting (37-41%, severe 4%)[13]; with SC use (0%)[3]
hemorrhage	GI hemorrhage (<1%)[13]
	hemoptysis (1%, severe 0%)
	CNS hemorrhage (<1%)[13]
	purpura (8%)[13]
hepatobiliary/pancreas	liver dysfunction (1%, severe 0%)
	pancreatitis (<1%)[13]
infection	**bacterial** (34-40%)[10]; usually occurs within 8 weeks, mycobacterial and tuberculosis have occurred months later[10] bacteremia without sepsis (4%)[24] *Listeria monocytogenes* meningitis (1%, severe 1%) *Pneumocystis* pneumonia (PCP)
	fungal (18-21%)[10]; majority occur within 3 months of treatment, late infection have occured[10] *Aspergillosis* (2%, severe 1%) candidiasis (8-10%, severe 1%)[13] *Cryptococcal* pneumonia (1%, severe 1%) mucormycosis (2%, severe 2%) *Torulopsis* pneumonia (1%, severe 1%)

Samsung Bioepis Exhibit No. 1002
Page 1073

ORGAN SITE	SIDE EFFECT
Clinically important side effects are in ***bold, italics***	
	not otherwise specified (7%, severe 1%)
	pneumonia (7-18%, severe 13%)[24]
	protozoan[7,24]
	sepsis (15-18%, severe 13%)[13]
	upper respiratory tract infection (6%, severe 0%)
	urinary tract infection (<3%, severe 0%)[24]
	viral (43-45%)[10] adenovirus[25] Cytomegalovirus (CMV) reactivation (4-66%)[3,10,14,15,24,26-28]; usually occurs between weeks 3-8 when T-cell numbers are low,[15] symptomatic CMV reactivations and infections (1-6%, severe 3%)[1,24,26,28] herpes simplex (10-13%, severe 1%)[13,24] herpes zoster (5%, severe 1%) Parvovirus B19 induced red cell aplasia (<1%)[29,30]
lymphatics	edema (1-13%, severe 0%)[13]
metabolic/laboratory	acidosis (<1%)[13]
	hypocalcemia (2%, severe 0%)
	hyponatremia (1%, severe 1%)
	increased alkaline phosphatase (<1%)[13]
	positive Coombs test without hemolysis (2%)
musculoskeletal	arthralgia (1%, severe 0%)
	myalgia (9-11%, severe 0%)[13]
neurology	anxiety (3%, severe 0%)
	confusion (2%, severe 1%)
	depression (2-7%, severe 0%)[13]
	dizziness (5-12%, severe 0%)[13]; vertigo (3%, severe 0%)
	leukoencephalopathy (<1%)[13]
	neurological symptoms associated with previous multiple sclerosis lesions; reversible, do not occur if pre-medicated with high dose steroids
	seizure (<1%)[13]
	sensory neuropathy; dysthesia (15%),[13] paresthesia (6%, severe 0%), hypoesthesia (3%, severe 0%)
	somnolence (3-5%, severe 0%)[13]
	tremor (6%, severe 0%)
ocular/visual	conjunctivitis (2%, severe 0%)
	endophthalmitis (1%, severe 1%)
	optic neuropathy (<1%)
pain	abdominal pain (6-11%, severe 2%)[13]

Samsung Bioepis Exhibit No. 1002
Page 1074

ORGAN SITE	SIDE EFFECT
Clinically important side effects are in ***bold, italics***	
	back pain (5%, severe 2%)
	chest pain (6-10%, severe 1%)[13]
	headache (18-24%, severe 1%)[13]; with SC use (0%)[3]
	SC injection site pain (61%, severe 2%)[3]
	not otherwise specified (7%, severe 1%)
	skeletal pain (3-24%, severe 1%)[13]
pulmonary	bronchitis (9%, severe 1%)
	bronchospasm (6%, severe 2%)
	cough (4-25%, severe 1%)[13]
	dyspnea (18-26%, severe 6%)[13]; with SC use (0%)[3]
	hypoxia (2%, severe 1%)
	pharyngitis (4-12%, severe 0%)[13]
	pneumonitis (3%, severe 3%)
	pulmonary infiltration (1%, severe 1%)
	respiratory depression (<1%)
	rhinitis (1-7%, severe 0%)[13]
	sinusitis (6%, severe 1%)
renal/genitourinary	hematuria (1%, severe 1%)
	decreased renal function/renal failure (<1%)[13,16]
sexual/reproductive function	binds to mature sperm
syndromes	influenza-like symptoms (5%, severe 0%)
	tumour lysis syndrome (<1%)[13]
vascular	phlebitis (<1%)
	vasospasm (1%, severe 0%)

Adapted from standard reference[1] unless specified otherwise.

Infusion reactions: Intravenous administration of alemtuzumab is usually accompanied by transient infusion-related side effects that manifest as flu-like symptoms. These acute cytokine-mediated reactions are generally mild to moderate. The reactions includes nausea and vomiting, hypotension, dizziness, rigors, fever, diarrhea, sudden sweating, shortness of breath, chills, and/or rash.[1,15] Serious and potentially fatal complications including myocardial infarction, cardiomyopathy, cardiac arrest, arrhythmias, bronchospasm, and acute respiratory distress have also been reported.[1,13] Symptoms are most common during the first week of therapy and decrease with each subsequent administration.[2,2,9,31] To prevent infusion related reactions:

- give diphenhydramine 50 mg PO and acetaminophen 650 mg PO 30 minutes prior to alemtuzumab[1,6]
- pre-medicate with prednisone 10 mg PO 15-30 minutes prior to alemtuzumab for the first 1-2 weeks[6,15]; since corticosteroids cause immunosuppression, prophylaxis should be discontinued after 1-2 weeks[15]
- start with low doses of alemtuzumab (3 mg) and escalate as tolerated[1] (for more information, see **Dosage Guidelines**); it is not known if the infusion reactions are strictly dose-related[6]

Samsung Bioepis Exhibit No. 1002
Page 1075

- meperidine 25 mg IV may lessen rigors,[6,9] patients experiencing meperidine-induced nausea should receive antiemetics[19]
- for patients experiencing severe rash, premedication with H_2 receptor antagonists (e.g., ranitidine) is recommended[19]
- fever commonly begins 5-6 hours after the infusion starts; a second dose of acetaminophen may be required[19]

During the infusion, the patient's pulse, respiratory rate and blood pressure should be monitored every 15 minutes during the first hour, then every 30 minutes.[6] Patients should be observed for 1 hour after the infusion.[6] To treat severe infusion-related symptoms, stop the alemtuzumab infusion and give hydrocortisone 200 mg IV.[1] Beta-2 agonists for wheezing and volume support for hypotension may be given.[32] If acute infusion reactions persist, the infusion time may be extended to up to 8 hours from the time of dose preparation.[31] Mild first-dose side effects tend to not preclude patients from completing therapy.[32]

Subcutaneous administration of alemtuzumab reduces infusion-related symptoms, while maintaining the same efficacy and improving convenience.[33] The incidence of nausea and vomiting, rigors, rash, hypotension, and dyspnea are significantly reduced with SC dosing, though fever may not be reduced appreciably (see Side Effects Table).[3] Almost all patients receiving SC alemtuzumab will develop a transient grade 1 or 2 local skin reaction.[3] IV corticosteroids do not affect the intensity or duration of these skin reactions.[3] Ice packs applied to the injection site 15 minutes prior to and 15 minutes post injection may decrease the severity of local skin reactions. Cold compresses applied to the injection site for 15-20 minutes, several times a day after treatment may also help alleviate symptoms. Most SC first-dose reactions disappear after 1-2 weeks.[3] Patients should be observed for 1 hour after the dose has been administered until at least three 30 mg doses have been given.[6] After that, monitoring may be discontinued.[6] Data from an ongoing study suggests that dose escalation may not be necessary with SC administration.[15]

Immunosuppression / opportunistic infections: Patients are at risk for opportunistic infections due to the profound lymphopenia induced by alemtuzumab.[1] Alemtuzumab-induced lymphopenia is non-dose dependant; single doses may reduce CD4+ and CD8+ T-cells for months.[15] Serious and sometimes fatal bacterial, fungal, viral and protozoal infections have occurred with infection rates ranging from 23-79% in pre-treated patients.[1,10] Although the majority of infections occur within 3 months of starting treatment, infections have been reported up to 6 months later.[10] If an infection occurs, alemtuzumab therapy should be interrupted but may be reinitiated following the resolution of the infection.[1]

Upon initiation of alemtuzumab patients should receive prophylaxis against PCP and herpes virus infections with:

- trimethoprim/sulfamethoxazole (TMP/SMX) DS PO twice daily three times weekly (TMP/SMX is the preferred prophylaxis; dapsone, pentamidine and atovaquone have also be used[10]
- acyclovir 400 mg PO BID (alternatively, valacyclovir 500 mg PO twice daily)[3]

Prophylaxis should be continued for a minimum of 2 months following the last dose of alemtuzumab or until CD4+ counts are ≥200 cells/μL, whichever occurs later.[1] Full recovery of CD4+ and CD8+ counts may take more than 12 months[1]; optimal length of post-treatment prophylaxis has yet to be determined.[10,15,19] Prophylaxis against PCP and herpes may decrease, but not eliminate, the occurrence of these infections.[1] Routine antifungal prophylaxis is not currently recommended but may be considered for high risk patients.[10,26]

Cytomegalovirus reactivation is the most common infection associated with alemtuzumab. T-cell depletion increases the risk of reactivation of CMV.[15,19] Reactivation usually occurs 3-8 weeks after initiation of therapy and coincides with T-cell nadir. Patients receiving alemtuzumab should have weekly testing for CMV replication by PCR or CMV antigenemia (pp65ag) assays.[6,11,19] Patients should also be monitored for symptoms of CMV infection.[6,19,34] If a positive screening test occurs, alemtuzumab treatment should be interrupted until testing becomes negative. Patients who are symptomatic should be treated immediately with ganciclovir.[10,14,15,19] Alemtuzumab has been restarted after clinical symptoms resolve and PCR testing is negative without further reactivation.[3,14,15,19]

Samsung Bioepis Exhibit No. 1002
Page 1076

INTERACTIONS:

AGENT	EFFECT	MECHANISM	MANAGEMENT
diagnostic serum tests that utilize antibodies[1,13]	inaccurate test results[1] and potential for increased risk of allergic reactions[13]	monoclonal antibodies may result in the formation of human antichimeric antibodies (HACA); the presence of HACA may increase the risk of hypersensitivity reactions when other monoclonal antibodies are administered[13]	interpret with caution
monoclonal antibodies[1,35]	increased risk of allergic or hypersensitivity reactions to monoclonal antibodies; especially in patients that experienced previous hypersensitivity reactions to monoclonal antibodies[1]	monoclonal antibodies may result in the formation of human antichimeric antibodies (HACA); the presence of HACA may increase the risk of hypersensitivity reactions, thrombocytopenia, or diminished therapeutic response when other monoclonal antibodies are administered; monoclonal antibodies that also form HACA may be of particular concern	monitor for toxicity/response, patients who developed hypersensitivity to alemtuzumab may develop hypersensitivity reactions to other monoclonal antibodies
killed virus vaccines[1]	ability to respond to vaccines following therapy is unknown; duration of decrease response unknown; estimates vary from 3 months - 1 year[9]	alemtuzumab may decrease the ability to generate a humoral response to vaccines	immunize prior to therapy if possible, potential for decreased benefit of vaccine if administered during or within 1 year after therapy
live virus vaccines[1]	ability to respond to vaccines following therapy is unknown, risk of infection by the live vaccine virus; duration of risk unknown; estimates vary from 3 months - 1 year[9]	alemtuzumab may potentiate replication of the vaccine virus, decrease the ability to generate a humoral response to the vaccine and enhance the adverse effects of live vaccines[9]	avoid during and within 1 year after therapy

SUPPLY AND STORAGE:

For basic information on the current brand used at the BC Cancer Agency, see Chemotherapy Preparation and Stability Chart in Appendix.

SOLUTION PREPARATION AND COMPATIBILITY:

For basic information on the current brand used at the BC Cancer Agency, see Chemotherapy Preparation and Stability Chart in Appendix.

Additional information: *Diluted solution for infusion*: compatible with PVC bags and administration sets[1]

Samsung Bioepis Exhibit No. 1002
Page 1077

Compatibility: consult detailed reference

PARENTERAL ADMINISTRATION:

BCCA administration guideline noted in ***bold, italics***

Subcutaneous	***rotate sites on thighs or abdomen****
Intramuscular	no information found
Direct intravenous	contraindicated
Intermittent infusion	***over 2 hours***
Continuous infusion	no information found
Intraperitoneal	no information found
Intrapleural	no information found
Intrathecal	no information found
Intra-arterial	no information found
Intravesical	no information found

*Subcutaneous administration of alemtuzumab significantly reduces flu-like symptoms making it the preferred route of administration.[12]

DOSAGE GUIDELINES:

Refer to protocol by which patient is being treated. Numerous dosing schedules exist and depend on disease, response and concomitant therapy. Guidelines for dosing also include consideration of absolute neutrophil count (ANC). Dosage may be reduced, delayed or discontinued in patients with bone marrow depression due to cytotoxic/radiation therapy or with other toxicities.

Adults:

BCCA usual dose noted in ***bold, italics***

	Cycle Length:	
Intravenous:	***titration 1 week***[1,6]**:**	***3 mg IV for one dose on day 1, then 10 mg IV for one dose on day 3, and 30 mg IV for one dose on day 5*** • **titration may be handled more slowly if required (e.g., infusion-related toxicities > NCI Grade 2)** • gradual dose escalation is required at the initiation of therapy and after treatment interruptions of 7 days or more; dose escalation to the recommended maintenance dose of 30 mg is required and can be accomplished in 3-7 days in most patients • doses greater than 30 mg per day or greater than 90 mg per week are not recommended
	maintenance 1 week[1,6]**:**	***30 mg (range 3-30 mg) IV for one dose on days 1, 3 and 5 (total dose per cycle) 90 mg (range 9-90 mg)*** • consecutive daily dosing may also be used[1] • gradual dose escalation is required after treatment interruptions of 7 days or more

Samsung Bioepis Exhibit No. 1002
Page 1078

BCCA usual dose noted in ***bold, italics***

Subcutaneous:

Cycle Length:

titration 1 week[3,6]***:*** ***3 mg SC for one dose on day 1, then 10 mg SC for one dose on day 3, and 30 mg SC for one dose on day 5***

- **titration may be handled more slowly if required (e.g., infusion-related toxicities > NCI Grade 2)**
- gradual dose escalation is required at the initiation of therapy; dose escalation to the recommended maintenance dose of 30 mg is required and can be accomplished in 3-7 days in most patients
- doses greater than 30 mg per day or greater than 90 mg per week are not recommended

maintenance 1 week[3,6]***:*** ***30 mg (range 3-30 mg) SC for one dose on days 1, 3 and 5 (total dose per cycle 90 mg [range 9-90 mg])***

- consecutive daily dosing may also be used[1]

Concurrent radiation: additive bone marrow suppression may occur; dose reduction may be required when used concurrently or consecutively[9]

Dosage in myelosuppression:[1] modify according to protocol by which patient is being treated; if no guidelines available, refer to the table below

ANC ($x10^9$/L)		Platelets ($x10^9$/L)	Dose
< 0.25	or	≤ 25	**first occurrence:** hold treatment and resume at the same dose when ANC ≥ 0.5 and platelets ≥ 50 (restart at 3 mg if delay between doses ≥ 7 days and escalate to 30 mg as tolerated) **second occurrence:** hold treatment and resume at 10 mg when ANC ≥ 0.5 and platelets ≥ 50 (restart at 3 mg if delay between doses ≥ 7 days and escalate to a maximum dose of 10 mg) **third occurrence:** discontinue treatment permanently
For baseline ANC ≤ 0.5 and/or platelets ≤ 25: if ANC or platelets decrease to ≤ 50% of baseline value, hold therapy and resume when ANC and platelet count return to baseline values (restart at 3 mg if delay between doses ≥ 7 days and escalate to 30 mg as tolerated)			
There is no information on the safety of resumption of alemtuzumab in patients with autoimmune cytopenias or marrow aplasia.[1]			

Dosage in renal failure: no information found

Dosage in hepatic failure: no information found

Dosage in dialysis: no information found

Children: has been used[36]

REFERENCES:

1. Berlex Canada. CAMPATH® product monograph. Pointe-Claire, Quebec; November 2005.
2. Keating MJ, Flinn I, V J, et al. Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. Blood 2002;99(10):3554-3561.

Samsung Bioepis Exhibit No. 1002
Page 1079

3. Lundin J, Kimby E, Bjorkholm M, et al. Phase II trial of subcutaneous anti-CD52 monoclonal antibody alemtuzumab (Campath-1H) as first-line treatment for patients with B-cell chronic lymphocytic leukemia (B-CLL). Blood 2002;100(3):768-773.
4. Rebello P, Hale G. Pharmacokinetics of CAMPATH-1H: Assay development and validation. Journal of Immunological Methods 2002;260(1-2):285-302.
5. Hale G, Rebello P, Brettman LR, et al. Blood concentrations of alemtuzumab and antiglobulin responses in patients with chronic lymphocytic leukemia following intravenous or subcutaneous routes of administration. Blood 2004;104(4):948-955.
6. B.C. Cancer Agency Lymphoma Tumour Group. (ULYALEM) BCCA protocol summary for the treatment of fludarabine-refractory B-chronic lymphocytic leukemia (B-CLL) and T-prolymphocytic leukemia (T-PLL) with alemtuzumab. Vancouver, British Columbia: BC Cancer Agency; 01 September 2006.
7. McEvoy GK, editor. AHFS 2006 Drug Information. Bethesda, Maryland: American Society of Health-System Pharmacists, Inc.; 2006. p. 923-925.
8. Dearden CE, Matutes E, Cazin B, et al. High remission rate in T-cell prolymphocytic leukemia with CAMPATH-1H. Blood 2001;98(6):1721-1726.
9. USPDI® Drug Information for the Health Care Professional (database on the Internet). Alemtuzumab (Systemic). Thompson MICROMEDEX®, 2006. Available at: www.micromedex.com. Accessed 19 July 2006.
10. Thursky KA, Worth LJ, Seymour JF, et al. Spectrum of infection, risk and recommendations for prophylaxis and screening among patients with lymphoproliferative disorders treated with alemtuzumab*. Br J Haematol 2006;132(1):3-12.
11. Judy Sutherland MD. Personal communication. Medical Oncologist, BCCA Lymphoma Tumour Group; 11 October 2006.
12. Joseph Connors MD. Personal communication. Chair, BCCA Lymphoma Tumour Group; 13 September 2006.
13. Rose BD editor. Alemtuzumab. UpToDate 14.1 ed. Waltham, Massachusetts: UpToDate; 18 July 2006.
14. Laurenti L, Piccioni P, Cattani P, et al. Cytomegalovirus reactivation during alemtuzumab therapy for chronic lymphocytic leukemia: incidence and treatment with oral ganciclovir. Haematologica 2004;89(10):1248-1252.
15. Osterborg A, Karlsson C, Lundin J, et al. Strategies in the management of alemtuzumab-related side effects. Semin Oncol 2006;33(2 Suppl 5):S29-35.
16. Basquiera AL, Berretta AR, Garcia JJ, et al. Coronary ischemia related to alemtuzumab therapy. Ann Oncol 2004;15(3):539-540.
17. Damaj G, Rubio MT, V A, et al. Severe cardiac toxicity after monoclonal antibody therapy. Eur J Haematol 2002;68(5):324.
18. Osborne WL, Lennard AL. Acute renal failure and disseminated intravascular coagulation following an idiosyncratic reaction to Alemtuzumab (Campath 1H) or fludarabine. Haematologica 2005;90(1):ECR05.
19. Keating M, Coutre S, Rai K, et al. Management guidelines for use alemtuzumab in B-cell chronic lymphocytic leukemia. Clinical Lymphoma 2004;4(4):220-227.
20. Margaret Gagnon. Personal communication. Medical Services Associate, Berlex Canada; August 2006.
21. Montillo M, Cafro AM, Tedeschi A, et al. Safety and efficacy of subcutaneous Campath-1H for treating residual disease in patients with chronic lymphocytic leukemia responding to fludarabine. Haematologica 2002;87(7):695-700.
22. Kunz JS, Banerji R, Kunz JS, et al. Alemtuzumab-induced syndrome of inappropriate anti-diuretic hormone. Leuk Lymphoma 2005;46(4):635-7.
23. BC Cancer Agency. (SCNAUSEA) Guidelines for Prevention and Treatment of Chemotherapy-induced Nausea and Vomiting in Adults. Vancouver, British Columbia: BC Cancer Agency; 1 November 2005.
24. Martin SI, Marty FM, Fiumara K, et al. Infectious complications associated with alemtuzumab use for lymphoproliferative disorders. Clin Infect Dis 2006;43(1):16-24.
25. Cavalli-Bjorkman N, Osby E, Lundin J, et al. Fatal adenovirus infection during alemtuzumab (anti-CD52 monoclonal antibody) treatment of a patient with fludarabine-refractory B-cell chronic lymphocytic leukemia. Medical Oncology 2002;19(4):277-280.
26. Nosari A, Molenti A. Risk of infections when using new therapeutic approaches for chronic lymphocytic leukemia. Haematologica 2003;88(suppl. 17):43-49.
27. Nosari A, Montillo M, Morra E. Infectious toxicity using alemtuzumab. Haematologica 2004;89(12):1415-1419.
28. Sandherr M, Einsele H, Hebart H, et al. Antiviral prophylaxis in patients with haematological malignancies and solid tumours: Guidelines of the Infectious Diseases Working Party (AGIHO) of the German Society for Hematology and Oncology (DGHO). Ann Oncol 2006;17(7):1051-1059.
29. Crowley B, Woodcock B. Red cell aplasia due to parvovirus B19 in a patient treated with alemtuzumab [7]. British Journal of Haematology 2002;119(1):279-280.
30. Herbert KE, Prince HM, Westerman DA. Pure red-cell aplasia due to parvovirus B19 infection in a patient treated with alemtuzumab [2]. Blood 2003;101(4):1654.
31. MARTINDALE - The Complete Drug Reference (database on the Internet). Alemtuzumab. Thomson MICROMEDEX®, 2006. Available at: http://www.micromedex.com/. Accessed 19 July 2006.
32. Flynn J, Byrd J. Campath-1H monoclonal antibody therapy. Curr Opin Oncol 2000;12(574-581).
33. Stilgenbauer S, Winkler D, Krober A, et al. Subcutaneous Campath-1H (Alemtuzumab) in Fludarabine-Refractory CII: Interim Analysis of the CLL2h Study of the German CII Study Group (GCLLSG). ASH Annual Meeting Abstracts 2004;104(11):478.
34. Schaal AD. Alemtuzumab (Campath 1-H). Clin J Oncol Nurs 2005;9(5):630-2.
35. Rose BD editor. Alemtuzumab. UpToDate 14.1 ed. Waltham, Massachusetts: Lexi-Interact ™ Online; 18 July 2006.
36. Shenoy S, Grossman WJ, DiPersio J, et al. A novel reduced-intensity stem cell transplant regimen for nonmalignant disorders. Bone Marrow Transplant 2005;35(4):345-352.

Samsung Bioepis Exhibit No. 1002
Page 1080

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Formalities officer: Mr Consalvo Cavestri

Please specify the name of the formalities officer when contacting Customer Services by contact form or phone.

Vossius & Partner
Siebertstrasse 4
81675 München
ALLEMAGNE

Date: 01-07-2014

Reference	Application No./Patent No.
M2367 EP	05712607.0 - 1412 / 1720571

Applicant/Proprietor: ALEXION PHARMACEUTICALS, INC.

Decision revoking the European patent (Art. 101(2), (3)(b) EPC)

The Opposition Division - at the oral proceedings dated 15.05.14 - has decided:

The European Patent No. 1720571 is revoked.

☐ Additional decision:
The opposition of the opponent(s) _____ is rejected as inadmissible.
Apportionment of costs (Art. 104(1) EPC) as indicated in the grounds for the decision.

The reasons for the decision are enclosed.

Appeal
This decision is open to appeal. Attention is drawn to the attached text of Articles 106 to 108 EPC and Rules 97 and 98 EPC.

Opposition Division:

1st Examiner:	2nd Examiner:	Chairman:
Chapman R	Weiki M	Cilensek Z

Enclosures: Reasons for the decision (Form 2916, 10 pages)
Text of Articles 106 - 108 and Rules 97 - 98 EPC (EPO Form 2019)
☒ Minutes of oral proceedings

Europäisches Patentamt · European Patent Office · Office européen des brevets

Registered letter with advice of delivery
EPO Form 2331 12.07 (26/06/14) REVO

to EPO postal service: 26.06.14

1 Facts and Submissions

Reference is made to the following documents cited by the Opponent; the numbering will be adhered to for the rest of the proceedings.

D1 Hillmen et al., (2004) The New England Journal of Medecine, 350:552-9

D2 Jane Salodof MacNeil (2002) Medscape Medical News

D3 Hillmen et al., (2003) British Journal of Hematology 121 (Suppl. 1) 87-95, Abstract 257

D4 Hillmen et al., (2002) Blood. 100(11), Abstract 154

D5 Hillmen et al., (2003) Journal of The American Society of Hematology 102(11) Abstract 1858

D6 Rother et al., (2003) Molecular Immunology. 40, p 197, Abstract 54

D7 Rosse et al., (2004) American Society of Hematology, p 48-62

D8 Biotechnology News (2004) 24(30), p 10

D9 US 6,355,245

D10 Thomas et al., (1996) Molecular Immunology, Vol. 33, No. 17/18, p 1389-1401

D11 Drugs of the Future (2004) 29(7): 673-676

D12 Hillmen et al., British Journal of Haematology, 125 (Suppl. 1), 1-72, Abstract 5

D13 Kaplan, (2002) Current Opinion in Inv. Drugs, vol. 3, no. 7, pages 1017-1023

D14 Declaration of Leonard Bell (2014)

D15 Packaging insert/Datasheet from Eculizumab (Alexion, 2011)

1.1 European patent EP1720571 (hereinafter designated the patent) entitled "Method of treating hemolytic disease", was published in the official bulletin on 13.06.2012 and a corrigendum was issued on 24.10.2012. The Proprietor (hereafter designated 'P') is Alexion Pharmaceuticals, Inc., Cheshire, US.

Said patent is based on European application 05712607.0, filed on 03.02.2005 and claims the priority from US 10/771,552 filed on 03.02.2004. The International publication number is WO2005/074607.

1.2 Notice of opposition against the above-mentioned patent was received from the following party (hereinafter named the Opponent or 'O').

1. Pfizer Inc., 235 East 42nd Street, New York 10017, US (filed online with letter dated 12.03.2013, received on 12.03.2013).

1.3 The O requested the revocation of the patent as granted in its entirety on the basis of Articles 100(a)(b)(c) because the subject-matter granted was not novel, inventive, or disclosed in a manner sufficiently clear and complete to be carried out by a skilled person (Articles 52(1),54,56 and 83 EPC), and comprised subject-matter that extended beyond the application as originally filed (Article 123(2) EPC). The O filed D1-D12 therewith.

1.4 In their letter dated 07.10.2013, the O withdrew their request of opposition against the above-mentioned patent.

1.5 With their letter dated 09.10.2013, the P requested that their patent be maintained as granted and that the opposition procedure be closed. The P requested oral proceedings in case their respective request would not be granted.

1.6 The Opposition Division (OD) issued a non-binding preliminary opinion with their official communication of 14.01.2014, based on the grounds of opposition raised by the O, and which the OD considered pertinent to pursue of its own motion, in accordance with Article 114 EPC. Oral proceedings were appointed for 15.05.2014.

1.7 The P submitted document D13 and arguments in support of maintenance of the patent as granted with their letter of 30.12.2013.

1.8 The OD reissued their non-binding preliminary opinion in order to account for the P's arguments that had not been presented to the OD before issuance of the summons to oral proceedings. The date scheduled for oral proceedings was maintained.

1.9 In their letter of 17.013.2014, the P submitted further arguments, a new main request (MR) and an auxiliary request (AR1) to replace the claims of the opposed patent. The P requested maintenance of the scheduled oral proceedings, in case the OD still not consider that any of the new requests overcome the objections raised.

1.10 Oral proceedings took place on 15.05.2014 in the presence of the P alone, since the O had withdrawn from proceedings. At the proceedings the Chairman (C) informed the P that the examination of the opposition was thus being continued of the OD's own motion. The P maintained their requests on file and submitted a declaration (D14), and a datasheet (D15) to support the subject-matter claimed.

1.11 At the end of oral proceedings, the OD announced the decision to revoke the patent in full in the absence of further allowable requests, because AR1 did not fulfill the requirements of Article 56 EPC.

2 Reasons for the Decision

Admissibility

2.1 The opposition complied with Articles 99 and 100 EPC. No objections were raised. The opposition is allowable.

Procedural matters

2.2 The O formally withdrew their opposition to the patent on 07.10.2013.

2.3 The OD considered that the arguments and documents submitted by the O to be valid grounds for opposition and thus decided to pursue opposition *proceedings of its own motion* (Article 114(1) EPC; GL D, VII-5.2;5.3).

2.4 The P filed D14, a declaration of a technical expert, and D15, a datasheet, during oral proceedings. The OD decided to admit D14 and D15 in to the proceedings, since they provided background information to the subject-matter claimed.

The decision is being carried out on the **following requests:**

Main Request

Description, Pages

3-13	of the patent specification			

Claims, Numbers

1-8	received on	18-03-2014	with letter of	18-03-2014

Drawings, Sheets

1/11-11/11	of the patent specification

Auxiliary Request 1

Description, Pages

3-13 of the patent specification

Claims, Numbers

1-8 received on 18-03-2014 with letter of 18-03-2014

Drawings, Sheets

1/11-11/11 of the patent specification

Main Request (MR)

The MR differs from the patent specification through the modification of the route of administration to explicitly state 'intravenous infusion', thus replacing the term 'intravenously' in claim 1 of the granted patent.

Rule 80, Articles 84, 123(2) and 123(3) EPC

2.5 The OD decided that the MR complies with Rule 80, Articles 84, 123(2) and 123(3) EPC since the term 'intravenous infusion' is clearly stated as the route of administration in the example on which it is based (p.32, li.13) in the application. The OD found that it would be obvious that all other steps in the claim comprising the same substance would be administered via the same route unless otherwise stated in the example. All objections raised were thus overcome.

Lack of novelty (Articles 100(a) and 54 EPC)

2.6 Objections were raised on grounds of lack of novelty of claims 1 and 5 with regard to D1.

2.7 D1 published on 05.02.2004 discloses all the features of claims 1-8 (see Methods and section 'Biochemical indicators of hemolysis' on p.552-556 and Fig.3A) but is published after the date of priority of the patent.

2.8 The patent claims priority from US10/771552, filed on 03.02.2004, and appears to disclose identical subject-matter to the application filed on 03.02.2005 on which the patent is based.

2.9 The OD considered that since the priority document discloses the claimed dosage regimen for treatment of complement breakthrough via intravenous infusion, D1 is not considered prior art and the subject-matter of claims 1 and 5 is new.

Lack of inventive step (Articles 56 and 100(a) EPC)

2.10 The OD objected to the MR on grounds of a lack of inventive step, in light of D5 alone as the closest prior art to the subject matter of claims 1 and 5, or in combination with any of D3, D4 or D6.

2.11 The P concurred that D5 should be considered the closest prior art, as it is directed to the same technical purpose, i.e. it is directed to treating a patient afflicted with PNH suffering a breakthrough in hemolytic activity.

2.12 Document D5 discloses the continuation of a pilot study comprising:

1. the use of eculizumab
2. the treatment of a patient affected with PNH
3. intravenous infusion
4. administration of 900mg bi-weekly
5. reducing the dosing interval slightly in response to breakthrough and thus resolving the symptoms of PNH.

2.13 The P stated that the difference between claim 1 and document D5 can therefore be considered to be that D5 does not disclose a complete dosage schedule. D5 is directed only to the maintenance stage of treatment, i.e. stages (iii) and (iv) of claim 1, and does not teach the essential induction phase stages (i) and (ii).

2.14 The technical effect associated with this difference is a complete dosage schedule for treating PNH and modifications for the alleviation of symptoms associated with a breakthrough in hemolytic activity.

2.15 The problem to be solved can therefore be considered to be the provision of a complete dosage regimen for maintaining patients with PNH symptom free.

2.16 The solution provided in the granted claims is not obvious, since the essential pre-loading stage is not taught in D5.

2.17 The P referred to the declaration D14, §4, wherein it is stated that the administration of 900 mg eculizumab at week five is essential for achieving an adequate baseline of complement inhibition that can be maintained with bi-

weekly 900 mg eculizumab doses thereafter. The P referred to the dosage regimen and provisions for its use, stated in the packaging datasheet D15 that accompanies eculizumab, as proof that this is the regimen used in practice.

The P thus concluded that D5 alone does not teach the features necessary to provide a complete dosage regimen for maintaining patients with PNH symptom free.

2.18 Furthermore, the P stated that D5 combined with any of D3,D4 or D6 would *not permit the skilled person to arrive at the regimen claimed*:

Documents D3 and D4 disclose a pilot study comprising the following features:

1. the use of eculizumab
2. the treatment of a patient affected with PNH
3. intravenous infusion
4. administration of 600mg each week for 4 weeks
5. administration of 900mg bi-weekly thereafter

The skilled person combining the teaching of D5 with any of D3 or D4 would not arrive at the dosage regimen claimed, since the administration of 900 mg eculizumab at week 5 is not taught.

2.19 The P explained that the skilled person would not arrive at the claimed dosage *regimen when combining the teaching of D5 with D6*:

Document D6 discloses the following features:

1. the use of eculizumab
2. the treatment of a patient affected with PNH
3. intravenous infusion
4. administration of 600mg each week for 4 weeks
5. administration of 900mg a week later
6. administration of 900mg bi-weekly thereafter

2.20 D6 teaches the administration of 900 mg eculizumab at week 5. However, the skilled person following the dosage schedule of D6 and experiencing breakthrough of hemolytic activity in PNH patients would not reduce the dosage interval to 900 mg eculizumab every 12 days: D5 merely teaches the 'slight' reduction of the dose in this situation. The P claimed that a reduction in dosage interval from 14 to 12 days cannot be considered slight, as it amounts

to around 15%. Moreover, the skilled person with a knowledge of D13 (p. 1021, col.1, §1 - 4) would know that the behaviour of the complement system is difficult to predict, and would not reduce the intervals by so much, and certainly not to 12 days, since shortening of dosing intervals may not result in an effective treatment by eculizumab.

2.21 The OD argued that the replacement of one regimen with another is a matter of routine experimentation, as supported by case law of the Technical Board of Appeal T1979/09, wherein under point 7.3.1 it is stated *'It is well known that in designing dosage regimen the two major parameters that can be adjusted are the dose itself, i.e. the quantity of drug administered, and the dosing frequency, i.e. the time interval between doses.'*

and further under point 7.3.2, *'In the present case the amount of active ingredient remains the same as the amount used in D5 and there is no evidence on file of any unexpected technical effect caused by administering the red vine leave extract once daily. The board considers that it is within the competence of the skilled person to find out the optimal dosage regime. Consequently, the replacement of a dosage regime by another dosage regime for the same purpose is considered to be a matter of routine experimentation and cannot be seen as involving an inventive step.'*

2.22 The P disagreed with this interpretation, since the present regimen comprises 4 different steps as stated in the claim, and thus cannot be compared to the simple regimen considered in T1979/09.

2.23 The OD argued that D13 is not directed to the same technical problem posed. Claim 1 is clearly directed to patients suffering breakthrough who are already receiving a standard regimen, as disclosed in D6. D5 clearly discloses how cases in which breakthrough is experienced can be resolved. D5, alike D6 was published after D13 and represents the actual stand of knowledge at the time of priority filing. The OD therefore considers that given the nature of D13 in reporting preliminary research results, and the subsequent publications D5 and D6, the skilled person would not consider D13 when selecting a dosage regimen for the indication presently claimed.

2.24 The P argued that neither D5 alone or combined with D6 any other cited document would anticipate the subject-matter of claim 1, however, if the skilled person were to combine D5 with other documents, he would select D3 or D4 as these relate to less complicated teachings.

2.25 The OD considers D6 to be the most recent and detailed teaching of the cited prior art D3,D4 and D6. The skilled person would therefore not consider D6 too complicated, rather to be the most up-to-date teaching for the treatment of PNH based on the pilot studies described in D2,D3 and D4.

2.26 The OD finds that according to D5, transient breakthroughs in complement activity associated with increased LDL, hemoglobinuria and mild symptoms in an extension trial of patients receiving the dosage regimen described in D6, were known to be preventable by a slight decrease in the dosing interval. The skilled person would have chosen and combined the teachings of D5 and D6 because they both describe the treatment of PNH with eculizumab. D6 discloses the latest details of a pilot study comprising the dose loading stages of parts (i) - (iii) of claim 1, whereas D5 describes an extension to what appears to be the same pilot study, wherein patients experiencing breakthrough of hemolytic activity are treated by a slight reduction in dosing interval, which successfully resolves their symptoms.

2.27 The OD thus concludes that the skilled person would be taught by D5 to reduce the dosage interval to solve the problem of breakthrough in PNH patients receiving the priming dosage regimen described in D6. The skilled person would reduce the interval 'slightly', i.e. by at say least 10%, to 12 days to resolve the symptoms, as suggested by D5.

The subject-matter of the MR therefore does not meet the requirements of Article 56 EPC.

Auxiliary Request 1 (AR1)

AR1 differs from the MR in that a result to be achieved by the dosage regimen of claim 1 of the MR has been added, i.e. a reduction of serum hemolytic activity (cell lysis) below 20%.

Rule 80, Articles 84, 123(2) and 123(3) EPC

2.28 The OD decided that AR1 complies with Rule 80, Articles 84, 123(2) and 123 (3) EPC since the term 'intravenous infusion' is clearly stated as the route of administration in the example on which it is based (p.32, li.13) in the application. The OD found that it would be obvious that all other steps in the claim comprising the same substance would be administered via the same route unless otherwise stated in the example. The feature that cell lysis is reduced by at least 20% was deemed to find a basis on p.38, li.11-17.

Lack of inventive step (Articles 56 and 100(a) EPC)

2.29 The OD objected to AR1 on grounds of a lack of inventive step, in light of D5 as the closest prior art to the subject matter of claims 1 and 5, in combination with D6.

2.30 The P argued that neither D5 nor D6 refer to any threshold value to be used to assess breakthrough of hemolytic activity.

2.31 The problem to be solved may thus be considered to be the provision of a dosage regimen that permits the maintenance of complement activity below a threshold that prevents breakthrough.

2.32 The solution is the dosage regimen of AR1. Neither D5 or D6 teach how to assess breakthrough by the parameters claimed. The application clearly discloses how serum hemolytic activity can be used to identify patients experiencing breakthrough, and that reducing this activity below 20% correlates resolves the symptoms of PNH (p.38 and Fig.8).

2.33 The OD noted that Fig.8 shows at least 4 patients with hemolytic activity above 20%, yet only 2 are described as experiencing breakthrough. The claimed parameter would thus appear to be a non-technical feature of the claim whose relevance is unclear, since patients with serum hemolytic activity above this threshold do not necessarily appear to demonstrate breakthrough.

2.34 The P explained that classic symptoms such as urine colour (hemoglobinuria) were signs of hemolytic breakthrough. In D5, LDH, hemoglobinuria and other mild symptoms were measured, but these are not direct methods of measuring serum complement activity. The parameter claimed thus identifies patients that are successfully treated.

2.35 The OD finds that the technical effect of the dosage regimen claimed is the treatment of PNH. The claim is directed to a dosage regimen and not to a method of diagnosis.

Fig.8 of the application demonstrates that patients with 0 - 30% serum hemolytic activity do not exhibit symptoms of PNH in the group tested. Not all patients are maintained below 20% activity, suggesting that the regimen claimed may not even achieve the technical effect claimed.

Furthermore, alternative methods for monitoring complement breakthrough are disclosed in Fig.4 of the application, which are the same as those used in D5.

The parameter of reducing hemolytic activity below 20% does not appear to provide a further technical feature associated with any surprising technical effect, but may at best be another read-out to confirm the same resolution of breakthrough already recorded in D5.

2.36 The OD therefore concludes that AR1 does not meet the requirements of Article 56 EPC.

Decision

3 Taking into account the amendments made by the P, the patent according to the Main Request or Auxiliary Request 1 was found not to comply with the requirements of Article 56 EPC. The OD decided to revoke the amended patent in accordance with the provisions of Article 101(3)(b) EPC.

T1697/14-3.3.04
(05 71 2607.0 / 1720571)
Patentee: Alexion Pharmaceuticals, Inc.
Opposition by: Pfizer Inc.
Our Ref.: M2367 OPP(EP)

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

15 July 2010
EMA/HMPC/16633/2009
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Vitis vinifera* L., folium

Based on Article 10a of Directive 2001/83/EC as amended (well-established use)

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Vitis vinifera* L., folium
Herbal preparation(s)	Herbal substance - Not applicable Herbal preparations: - Comminuted dried leaves as herbal tea (TU) - Powdered herbal substance (TU) - Soft extract (2.5-4:1; water (TU) - Dry extract (4-6:1; water) (WEU)
Pharmaceutical forms	Comminuted herbal substance as herbal tea for oral use (TU). Herbal preparation in solid dosage forms for oral use (WEU and TU). Herbal preparation in semi-solid dosage forms for cutaneous use (TU).
Rapporteur	Dr Ioanna Chinou, Gloria Garcia-Llorente

7 Westferry Circus • Canary Wharf • London E14 4HB • United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7523 7051
E-mail info@ema.europa.eu **Website** www.ema.europa.eu

An agency of the European Union

© European Medicines Agency, 2010. Reproduction is authorised provided the source is acknowledged.

Table of contents

Table of contents 2

1. Introduction 4

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof . 4

1.2. Information about products on the market in the Member States 5

2. Historical data on medicinal use 11

2.1. Information on period of medicinal use in the Community 11

2.2. Information on traditional/current indications and specified substances/preparations . 14

2.3. Specified strength/posology/route of administration/duration of use for relevant preparations and indications 15

3. Non-Clinical Data 15

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof 15

3.1.1. Assessor's overall conclusions on pharmacology 23

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof 23

3.2.1. Assessor's overall conclusions on pharmacokinetics 23

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof 24

3.3.1. Assessor's overall conclusions on toxicology 26

4. Clinical Data 26

4.1. Clinical Pharmacology 26

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents 27

4.1.1.1. Assessor's overall conclusions on pharmacodynamics 27

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents 27

4.1.2.1. Assessor's overall conclusions on pharmacokinetics 28

4.2. Clinical Efficacy 28

4.2.1. Dose response studies 28

4.2.2. Clinical studies (case studies and clinical trials) 29

4.2.3. Clinical studies in special populations (e.g. elderly and children) 35

4.3. Overall conclusions on clinical pharmacology and efficacy 35

5. Clinical Safety/Pharmacovigilance 36

5.1. Overview of toxicological/safety data from clinical trials in humans 36

5.2. Patient exposure 36

5.3. Adverse events 36

5.4. Serious adverse events and deaths 38

5.5. Laboratory findings 38

5.6. Safety in special populations and situations 38

5.7. Intrinsic (including elderly and children) /extrinsic factors 38

5.8. Drug interactions 39

5.9. Use in pregnancy and lactation 39

5.10. Overdose 39

5.11. Drug abuse 39

Samsung Bioepis Exhibit No. 1002
Page 1093

5.12. Withdrawal and rebound 39
5.13. Effects on ability to drive or operate machinery or impairment of mental ability 39
5.14. Overall conclusions on clinical safety 39
6. Overall conclusions 39
Annex 40

Samsung Bioepis Exhibit No. 1002
Page 1094

1. Introduction

The aim of this report is to assess the preclinical and clinical available data on Vitis viniferae folium for preparing a Community herbal monograph and Community List entry. This report is based on the documentation provided by the European Medicines Agency (EMA) completed by additional searches and information taken from recent international literature on Vitis viniferae folium.

Bibliographic searches have been performed in MEDLINE (1966-2008); EMBASE (1974-2008). The search terms were: Plant extracts; Plant leaves; Red vine leaf extract; Roter Weinlaubextrakt; Extract Red vine / wine Leaf / Leaves *Vitis vinifera*; Folia *Vitis vinifera.*
Only preparations of *Vitis vinifera* as a single ingredient are considered for the monograph, while the studies performed with combinations are not discussed in this report.

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

The plant originates from North Africa, South Africa or South West Europe. *Vitis vinifera* L., Grape vine, is a perennial, defoliating limber with a wooden often twisted stem which can reach a length of 30 meters, but it is usually cut back to 1-3 meters. The shrub develops climbing branches forking to twigs from where the long-stemmed, alternating arranged leaves protrude. The vine leaf is heart-shaped, thin, long-stemmed, cordate with palmate venation and with 5-7 dentate lobes, divided by more or less deep and open sinuses. It can be up to 15 cm long and 12 cm across at its widest point, its colour is a uniform dark red. The upper surface is glabrous, but the lower surface may be pubescent. The venation is prominent on the lower surface. At the lower tendrils the flower panicles with numerous yellow-greenish flowers are formed. The fruits, arranged in large and long clusters are soft and pulpy berries with yellow-green, reddish or purplish dark-blue skin [Pharm. Franc. X., 1996].
Vitis vinifera belongs to the *Vitaceae* family. Several subspecies and varieties are distinguished among which is the subspecies *sylvestris* (Gmelin) Berger, recognised as the spontaneous form of *V. vinifera* L., the subspecies *caucasica* Vavilov, occurring in both wild and cultivated form. It is supposed that from these two, the cultivated form *Vitis vinifera* ssp. *sativa* DC has been grown [Bombardelli & Morazzoni 1995, Bombardelli et al. 1997].

- Herbal substance

The herbal substance consists of the dried leaves of the black to pulp-red grape and has a faintly perceptible odour. The herbal drug is harvested by hand in the autumn following the grape harvest. Drying takes place under natural conditions in accordance with local climatic conditions.

Latin Name: Vitis viniferae folium (*Vitis vinifera* L., var. *tinctoria*, *Vitaceae*) "Vitis vinifera" or "Vitis folium";
Common Names: vine leaves or vineleaves or vine leaf or vineleaf or grape leaf (English);
Feuilles de vigne (French), Rebenblätter, Weinlaub (German), Fogli della vite (Italian), Hogas de la vid (Spanish), Folhas da videira (Portuguese), Wijnstok bladeren (Dutch), liść winorośli właściwej (Polish), Φύλλο Αμπέλου (Greek).

The crude herbal substance complies with the monograph "Vigne Rouge" French Pharmacopoeia [Pharm. Franc. X., 1996]. The powder is reddish-brown. Examined under the microscope, the powdered red vine leaf shows the following characteristics: more or less dense, unicellular, long, tapering, covering trichomes, thick-walled, with bulbous or truncated base and a lumen divided into loculi; numerous raphides of calcium oxalate are contained in these cells or scattered about; fragments

Samsung Bioepis Exhibit No. 1002
Page 1095

of parenchyma containing twinned crystals of calcium oxalate; a few fragments of epidermis with polygonal cells and some reticulate venation.
Since the beginning of the last century in many studies the chemical constituents of the different parts of grape vine have been investigated. Fruit acids, tannins and pigments are the substances mainly responsible for taste, odour and colour [PDR for Herbal Medicines, 2004].

- Herbal preparation(s)

Comminuted herbal substance

Powdered herbal substance

Soft extract (DER 2.5-4:1); extraction solvent water

Dry extract (4-6:1; water)

See also market overview in 1.2

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

Not applicable.

1.2. Information about products on the market in the Member States

Member State	**Regulatory Status**	**Comments**[1]
Member state	products, indications	Legal status
Austria	1) **360 mg-filmcoated tablet** 1 tablet contains: 360 mg dry extract (DER 4-6:1), extraction solvent water 2) **capsules** 1 capsule contains: 180 mg dry extract (DER 4-6:1), extraction solvent water 3) **oral drops** 1 ml contains 73.5 mg extract (corresponding 1.2 mg Aesculin) in ethanolic solution (the DER and the extraction solvent are not declared in the SPC at the moment) Since 1) December 2005 2) January 2000 3) March 1993 1) 1 tablet daily (up to 2 tablets). Duration of use: max. 3 months 2) 2 capsules in the morning (up to 4 capsules). Duration of use: max. 3 months 3) 30 drops in the morning in fasting state **Indications** 1,2) chronic venous insufficiency grade I and II; swelling of feet and lower leg	WEU

[1] Not mandatory field

Samsung Bioepis Exhibit No. 1002
Page 1096

Member State	Regulatory Status	Comments[1]
	3) phlebitis, edemas in the legs (also during pregnancy), varicose veins	
Belgium	**Hard-capsules** 350 mg powder per capsule powder, corresponding to 8% total polyphenols and minimum 0.20% of anthocyanosides Since 2005 oral administration Adults: 1 to 2 capsules three times daily, during the meals with a large glass of water. Maximal dose is 6 capsules per day. Duration of use should be limited to 3 months. If symptoms do not improve during use, consult a doctor **Indications** used in case of subjective symptoms of venous insufficiency in legs, such as heavy legs, after exclusion of all serious pathologies such as phlebitis, thrombophlebitis and thrombosis	WEU
Bulgaria	Not known	
Cyprus	Not known	
Czech Republic	**(WEU)** Vitis viniferae folii extractum aquosum siccum (4–6:1), 180 mg – standardized to flavonoids content 3-7% **Pharmaceutical form** Capsule **Posology** 2 capsules corresponding to 360 mg of the extract in the morning, during first 3 weeks increase of the dose to 4 capsules is possible to reach faster relief since 2001?? **Indications** Prevention and therapy of symptoms of light or starting moderate forms of chronic venous insufficiency related to varicose veins such as swelling of the calves, heavy legs, tingling	
Denmark	No authorized herbal medicinal products containing *Vitis vinifera* as a single drug preparation are on the market.	
Estonia	Not known	
Finland	Not known	
France	**(WEU)** Soft extract since 1970 **Pharmaceutical form** eye drops **Posology** 1 drop 2 to 8 times daily. 1 g of extract/100 ml **Indications** uninfectious conjonctival irritations **TU** **Preparations** Powder since 1982 dry aqueous extract since 1987, 1990 All for oral use, in adults **Posology**	

Samsung Bioepis Exhibit No. 1002
Page 1097

Member State	Regulatory Status	Comments[1]
	1) 1 hard capsule 3 times daily (5 if necessary) 350 mg of powdered drug/capsule 2) 1hard capsule 2 times daily 200 mg of extract/capsule 3) 1 hard capsule 1 to 3 times daily containing 169 mg of extract/capsule **Indications** 1); 2); 3) - Traditionally used in the symptomatic treatment of functional disorders of cutaneous capillary fragility, such as ecchymosis, petechias, etc. - Traditionally used:, in subjective signs of venous insufficiency, such as heavy legs - in haemorrhoidal symptoms combination herbal preparations with Mélilot (*Melilotus*) Marron d'inde (*Aesculus hippocastanum*) Hamamélis (Hamamelis)	
Germany	**TU** **Preparations**: soft extract (2.5-4:1), extraction solvent: water at least since 1976 **Pharmaceutical form** cream **Posology** for cutaneous use in adults 10 g cream contain 282 mg soft extract **Indications** Traditionally used to relieve symptoms of tired legs **Risks** (adverse drug effects, literature) Allergic reactions, itching and erythema, worsening of skin irritation. Contact allergy and/or hypersensitivity reactions **WEU** 1) soft extract (4-6:1), extraction solvent: water 2), 3) dry extract (4-6:1), extraction solvent: water In the market 1), 3) at least since 1976 2) 2005 1) oral liquid 2) film-coated tablet 3) capsule, hard all for oral use in adults **Posology** 1) 1 x daily 6 ml liquid if necessary 1 x daily 12 ml liquid 10 ml (= 11.013 g) liquid contain 0.6 g soft extract 2) 1 x daily 1-2 containing 360 mg dry extract each 3) 1 x daily 2 containing 180 mg dry extract each if necessary 1 x daily 4 **Indications** For symptomatic treatment of chronic venous insufficiency, which is characterised by swollen legs, a feeling	

Samsung Bioepis Exhibit No. 1002
Page 1098

Member State	Regulatory Status	Comments[1]
	of heaviness, pain, itching, cramps in the calves at night **Risks** Common gastrointestinal complaints, nausea. Hypersensitivity reactions of the skin (itching and erythema, urticaria) have been reported	
Greece	No authorized herbal medicinal products containing *Vitis vinifera* as a single drug preparation are on the market	
Hungary	1)180 mg capsules 1 capsule contains: 180 mg dry extract (DER 4-6:1), extraction solvent water (2008) 2) oral drops 81.4 mg Vitis viniferae rubrae folii extr. spissum(4-6:1), corresponding to 1.2 mg aescin/ml (1997) 3) Varixinal capsules 180 mg dry native extract of *Vitis vinifera* L., folium (DER: 4-6:1) extraction solvent: water (2009) 1) 2 capsules once a day 2) 30 drops in the morning in fasting state 3) 2 capsules once a day **Indications** 1) chronic venous insufficiency; at stage of C_2, C_3 and C_{4A} according to CEAP 2); 3) For alleviating the discomfort feeling (fatigability, tension of the legs) of the lower extremities occurring in the course of the mild venous circulation disturbance 1); 3) It is not recommended during pregnancy and lactation. Adverse effects: Gastrointestinal disorders (Common) stomach and intestinal discomfort, nausea Skin and subcutaneous tissue disorders: (Uncommon) rash, itching, hives	
Iceland	Not known	
Ireland	Not known	
Italy		Food supplements only.
Latvia	**Preparations** *Vitis viniferae* folii extractum aquosum siccum (4–6:1) 180 mg – standardized to flavonoids content 3-7% since 2004 **Pharmaceutical form** Capsule **Indications** Prevention and treatment of signs related to chronic venous insufficiency	
Lithuania	Not known	
Luxembourg	Not known	
Malta	Not known	
Netherlands	Not known	

Samsung Bioepis Exhibit No. 1002
Page 1099

Member State	Regulatory Status	Comments[1]
Norway	Not known	
Poland	Vitis viniferae extractum siccum (5-7:1), extraction solvent: water, capsule hard Product allowed onto the market according to previous polish pharmaceutical low (less than 10 years). **Posology** oral use: adults, 2 capsules once a day (in the morning) **Indications** support of mild symptoms of chronic venous insufficiency	
Portugal	No authorized herbal medicinal products containing *Vitis vinifera* as a single drug preparation are on the market	One authorised combination product.
Romania	Not known	
Slovakia	**Preparations** Vitis viniferae folii extractum aquosum siccum (4–6:1) 180 mg – standardized to flavonoids content 3-7% since 2005 **Pharmaceutical form** Capsule **Posology** 2 capsules (360 mg) once a day, in the morning time. Total daily dose can be increased up to 4 capsules once a day, in the morning time **Indications** 1) Prevention and treatment of signs related to chronic venous insufficiency, which is usually expressed in connection with varicoses and oedema in the lower parts of legs, fatigue, pins and needles and pain in legs **Risks (adverse drug effects)** Allergic reactions including generalized rash and urticaria. Nausea, gastric complaints	
Slovenia	Not known	
Spain	**Preparations** **(WEU)** Vitis viniferae folii extractum aquosum siccum (4–6:1), 180 mg – standardized to flavonoids content 3-7% **Posology** 2 capsules (360 mg) once a day, in the morning time. Since 2001 **Indications** Prevention and treatment of signs related to chronic venous insufficiency, which is usually expressed in connection with varicoses and oedema in the lower parts of legs, fatigue, pins and needles and pain in legs **(TU)** Powdered herb. substance 270 mg/caps	
Sweden	No authorized herbal medicinal products containing *Vitis vinifera* as a single drug preparation are on the market	
United Kingdom	Not known	

Samsung Bioepis Exhibit No. 1002
Page 1100

Regulatory status overview

Member State	Regulatory Status				Comments (not mandatory field)
Austria	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	(4-6.1, water extr tab, 2005, 180 mg caps, 2003, drops 73.5 mg/ ml, corresp. 1.2 (aesculin), 1993
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	WEU Caps 350 mg 1- 6/d
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Czech Republic	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Ext aq (4-6:1) standardized 3-7% flavonoids. Caps 180 mg, 2001
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No product registered
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
France	☒ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	WEU, eye drops Aqueous soft extract (6:1) since 1970 TU, powder(1989) or water extr. for oral use (1987)
Germany	☒ MA	☐ TRAD	☒ Other TRAD	☐ Other Specify:	dry water extr. (4-6:1), 180mg 1976, (360 mg) 2005 oral liquid, 10 ml contain 0.6 g soft extr. **TU** soft water extr. (2.5-4:1), 1976, cream, 10 g contain 282 mg soft extr.
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No product registered
Hungary	☒ MA	☐ TRAD	☒ Other TRAD	☐ Other Specify:	(4-6.1, water extr tab, 2005, 180 mg caps, 2008 & 2009, drops 81.4 mg/ ml, corresponding. 1.2 (aesculin), 1997
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known

Samsung Bioepis Exhibit No. 1002
Page 1101

Member State	Regulatory Status				Comments (not mandatory field)
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Food Suppl. No product registered
Latvia	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	WEU Ext aq (4-6:1) standardized 3-7% flavonoids caps 180 mg, 2004
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Poland	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No product registered
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Slovak Republic	☒ MA	☐ TRAD	☒ Other TRAD	☐ Other Specify:	WEU Ext aq (4-6:1) standardized 3-7% flavonoids. Caps 180 mg, 2005
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Spain	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	WEU Ext aq (4-6:1) standard. 3-7% flavon. caps 180 mg Powdered herb. substance 270 mg/caps
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No product registered
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known

MA: Marketing Authorisation
TRAD: Traditional Use Registration
Other TRAD: Other national Traditional systems of registration
Other: If known, it should be specified or otherwise add 'Not Known'
This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

Red grapevine leaves, from *Vitis vinifera* L. [Fam. *Vitaceae*], are rich in flavonoids including anthocyanins, oligomeric proanthocyanidins (OPCs) etc.
The knowledge of the medicinal potential of grape vine *(Vitis vinifera)* can be traced back far in history:

Samsung Bioepis Exhibit No. 1002
Page 1102

- In Europe, the leaves of Vitis vinifera are documented in the literature of traditional medicine for their astringent and homeostatic properties where they are utilized in the treatment of diarrhoea, bleeding, haemorrhoids, varicose veins and other circulatory and venous diseases [Anonymous 2004; 2005; 2006; Bombardelli & Morazzoni 1995].
- In Turkish folk medicine, vine leaves are known to have a diuretic effect, while the juice of leaves have been used as an eye bath [Kosar et al. 2007].
- Native North American indigenous peoples used the leaf tea of related fox grape (*Vitis labrusca* L.) for treating diarrhoea, as well as for hepatitis, stomach aches and thrush and externally poulticed the wilted leaves for sore breasts, rheumatism, headaches and fevers. Other closely related Vitis species have been used similarly.
- In Indian Medicine: Grape is used for headache, dysuria, scabies, skin disease, gonorrhoea, haemorrhoids and vomiting [PDR for herbal Medicines 2004].

Since ancient times, beneficial effects on health have been ascribed to wine and vine leaves, as confirmed by numerous "recipes" reported in Egyptian papyri, the Sumerian tablets, the writings of Hippocrates of Cos (5th-4th century BC), Celsus (1st century AD), Galen (130-201 AD) and Paracelsus (1493-1541). The use of vine leaves has been documented. Retracing therapeutic literature from France showed the origin of using red leaves shortly after first incidence of the respective grape vine varieties called "teinturiers" [Schneider, 2007i]. The herbal substance and herbal preparations have a long history of use in folk medicine where French winegrowers collected the red vine leaves at the time of the grape harvest to make infusions and paste-like poultices from them. The infusions were filled into bottles and regularly ingested in small quantities. The paste from vine leaves was used for the treatment of swollen, painful legs. Nowadays, the extracts of grapevine leaves were developed into modern herbal medicaments mainly used against venous diseases and for its effects on microcirculation [Rabe et al. 2005; Volonté M, Petrini, 2004; Volonté et al. 2003]. The vine, fresh and brined or fermented are used as food (mixtures of rice and spices with or without meat are wrapped with vine leaves) and have been widely consumed as traditional foods (Dolmas) around the Mediterranean countries [Kosar et al. 2007]. They are also used for diarrhoea, vomiting and varicose treatment [Gharib Naseri & Heirari 2006].

There is evidence of the use of grapevine leaf outside France, in Italy in 1957, where the Biosedra preparation was also tested in vascular disorders in gynaecology, producing a positive effect on capillary fragility.

As early as 1960, a clinical study was published in Germany on the venous efficacy of a product, which contained a fluid extract of grapevine leaf, with a drug/extract ratio of 1:1. The content of anthocyanin was set at 600 µg/ml,which was subsequently changed to 1.2 mg bioflavonoids per ml (German *Rote Liste* 1974). In 1969, a company registered the preparation as a medicinal product, with the indications of varices, phlebitis, thrombophlebitis, calf cramps and leg oedema. The successor product, has been marketed since 1971. The capsules and tablets contain 180 mg and 360 mg of dried extract of grapevine leaves.

An overview of grapevine leaf components and its supposed pharmacological action as well as the polyphenol composition of *Vitis vinifera* can be found in the literature [Anonymous 2003; 2004; Petrini et al. 2003; Schneider, 2007i; 2007ii; Schneider et al. 2008; Schaefer & Red 2003].
Nonclinical *in vitro* and *in vivo* studies suggested protective effects of components from extracts of grapevine leaves on the venous system; e.g. procyanidines [Maffei et al. 1994; Constantini et al. 1999], and flavonoids [Nees et al. 2003]. For the extract preparation *(Extractum Vitis viniferae foliae aquosum siccum, 4-6:1)*, dried leaves of red varieties of *Vitis vinifera* L. (Vitis viniferae folium) which comply with the monograph described in Pharmacopée Français (10ème edition) for "vigne rouge" are

Samsung Bioepis Exhibit No. 1002
Page 1103

used. Thus, the herbal substance consists of the dried leaves of the black to pulp-red grape which finally undergo a specific production process resulting in a defined flavonol content in the dry extract preparation. While the whole dry extract preparation as such is considered as active agent, it is particularly characterized by its content of flavonol glycosides and glucuronides, i.e. quercetin-3-O-β-D-glucuronide, quercetin-3-O-β-glucoside, and kaempferol-3-glucoside. These flavonoids are considered to contribute predominantly to pharmacological effects. The extract AS 195 contains a total of 4-7% of flavonol glycosides, quantified as quercetin-3-O-β-D-glucuronide.

Grapevine leaves and extracts thereof have been traditionally used for the treatment of symptoms associated with venous insufficiency for more than 70 years, in France. Extracts have been introduced in a number of European countries, e.g. Austria, Belgium, Czech Republic, Spain, Switzerland, Italy, and the United Kingdom. It is indicated for the prevention and treatment of Chronic venous insufficiency (CVI), associated with varicose veins including oedema of the lower leg, heavy or tired legs, sensation of tension, tingling and pain.

Based on the survey of products available in the Member states, the traditional use of red leaves preparations has been demonstrated especially in France, Germany, Spain and Austria, for at least 30 years.

Red vine leaf has been included in the French official list entitled "Avis aux fabricants concernant les demandes d'Autorisation de mise sur le marché" by virtue of which, the products containing it were subjected to a simplified registration procedure before the implementation of the Directive 2004/24/EC.
In France: "Vigne Rouge" (= red vine leaf) is regarded as one of the herbal drugs whose efficacy and safety has been proven by thorough literature studies and long-term traditional use. The Pharmacopèe Française X ed. includes the monographs of "Vigne rouge" and "Extrait de vigne rouge (sec)" and the traditional use of red vine leaves is discussed in "Rotes Weinlaub" [Schneider, 2007i; 2007ii].

Herbal medicinal products with red vine leaf have a long tradition in France. The official compendium "Médicaments à base de plantes", issued by the French department "Ministère des Affaires sociales et de la solidarité", released 1990, and comprised the long tradition of diverse herbals in France. There are listed many herbals that have been used in France for a long time, at least for about 15-20 years. [Les Cahiers de l'Agence 1998] state the use of red vine leaf for symptomatic relief of chronic venous disease ("No 015+016: " Traditionellement utilise dans le traitement symtomatique des troubles fonctionnels de la fragilité capillaire cutanée, tels que ecchymoses, pétéchies, etc."; No. 017+018: "Traditionnellement utilisée: - dans les manifestations subjectives de l'insuffisance veineuse telles que jambes lourdes; - dans la symptomatologie hémorroïdaire").
Different products prepared of water extracts of "Vigne Rouge" (5:1) have been available in oral forms, in France to treat blood vessels fragility in order to reduce the feeling of heavy legs and haemorrhoids related disorders. The posology range from 169 mg to 200 mg, two to three times a day, for four weeks:

Vigne rouge, capsules: 1 capsule contains 200 mg of grapevine leaves dry aqueous extract is used, a DER of about (4-6:1). The product is registered since 1987. The manufacturer recommends the use of 2 capsules per day (1 capsule mornings and one evening, with a glass of water). The product is traditionally used to reduce fine blood vessels fragility, in order to reduce the feeling of heavy legs and haemorrhoids related disorders.
OR
1 capsule contains 169 mg of grapevine leaves dry extract (3:1). The manufacturer recommends the use of up to 3 capsules per day. The product is traditionally used to reduce the feeling of heavy legs, haemorrhoids related disorder and fine blood vessels fragility.

Samsung Bioepis Exhibit No. 1002
Page 1104

An article from 'Deutsches Medizinisches Journal' published in 1960 by K. Güthenke, "Wesen und Behandlung der Bindegewebsschwäche, insbesondere der Veneninsuffizienz mit Weinblattextrakt" (Nature and Treatment of weak connective tissues, in particular venous insufficiency, ref.) describes the long traditional use of grapevine leaves for treatment of diseases of the leg veins, and demonstrates that vine leaf extracts have been already traditionally used before 1960, in Germany. Registrations for the active ingredient *Extractum vitis viniferae foliae aquosum siccum* exist since 1969. Since 1974 a solution for oral use has been available. 20 ml contained 1-2 g of extract from leaves of *Vitis vinifera* (1:4-5) and 0.02 g of aesculin. In 1999 aesculin was removed from the formulation. The recommended dosage is 2-3 x 30 drops per day. The product is traditionally used to treat diseases of the leg veins (f.e. varicose veins, chronic venous insufficiency), to reduce pains and feeling of heavy legs, night time cramps in the calf, itching and swollen legs. The use of this product can be proven for over 30 years.

In 1991 the galenical form of hard gelatine capsules was registered and marketed for the first time. One capsule contained a combination of 180 mg of *Extractum vitis viniferae foliae aquosum siccum* and 3 mg aesculin (a coumarine glucoside that naturally occurs in the horse chestnut *Aesculus hippocastanum*). This herbal medicinal product was indicated for the treatment of venous insufficiency, varices, haemorrhoids, heavy tired legs and feet, essential oedema and calf cramps. The daily posology was 2-3 capsules a day. In 1999, aesculin was removed from the product and the only active substance declared was the dry extract of grapevine leaves. The capsules contain 180 mg of *Extractum vitis viniferae* (extraction solvent: water, DER: 4–6:1), with a recommended use of 2-4 capsules per day.
A cream (ointment) -containing soft extract (4-6:1; water) in a cream base
(10 g cream contain 282 mg soft extract)- has been traditionally used since 1976 to relieve symptoms of tired legs applying it several times daily.

Conclusions on traditional use
Based on information obtained from Member states and data retrieved from handbooks it can be concluded that the following extracts and uses of grapevine leaf extracts fulfil the criteria for traditional use:

Herbal substance

Not applicable.

Herbal preparations:

- Comminuted dried leaves
- Powdered dried leaf
- Soft extract (2.5-4:1; water) in a cream base

Concerning the preparation: dry extract (3:1; water), the product has been on the market since 1990 (France) and does not comply with the requirements of Directive 2004/24/EC for THMP. There is no complete information on the manufacturing process and DER of this preparation, and it is not possible to evaluate the similarity with other dry extracts under WEU.

2.2. Information on traditional/current indications and specified substances/preparations

Indication:

Traditional herbal medicinal product:

Samsung Bioepis Exhibit No. 1002
Page 1105

a) To relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances.

b) For symptomatic relief of itching and burning associated with haemorrhoids.

c) For symptomatic treatment of cutaneous capillary fragility.

The product is a traditional herbal medicinal product for use in the specified indication exclusively based upon long-standing use.

2.3. Specified strength/posology/route of administration/duration of use for relevant preparations and indications

Posology:

-Dried leaves in herbal teas, 40 g/l. One cup (10g/250 ml) 2-4 times/day [ESCOP 2009]
-Comminuted total powder: 270-350 mg, 3 times/day (before the meals), up to 2100 mg/day
-Soft extract (2.5-4:1; water), in a cream base, (10g cream contain 282 mg soft extract), a thin layer should be spread on the affected area 1-3 times/day

-Dry extract (DER 4-6:1; water), single dose: 360-720 mg, daily dose: 360-720 mg

3. Non-Clinical Data

Non-clinical *in vitro* and *in vivo* studies suggested protective effects of components from extracts of grapevine leaves on the venous system; e.g. procyanidines [Maffei et al. 1994; Constantini et al. 1999], and flavonoids [Nees et al. 2003].

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Constituents

Vine leaves contain a wide range of polyphenol flavonoids including flavon(ol)-glycosides and glucuronides, quercetin-3-O-beta-D-glucuronide (most abundant of flavonoids), isoquercitrin, anthocyanins, oligomeric proanthocyanidins [Hmamouchi et al. 1997], catechin, epicatechin monomers and dimmers and gallic acid. The phytoalexin trans-resveratrol, another polyphenolic substance belonging to the stilbene group, can also be found in grape vine [Langcake et al. 1979]. It has been referred in the international literature that $AlCl_3$ is a potent elicitor of resveratrol production in vine leaves [Andrian et al. 1996]. In vine leaves, also organic acids appear, mainly malic and oxalic acid but also tartaric acid. Citric, fumaric and succinic acid can be detected in the leaves only in traces. Compared to the grape berries, grape leaves are richer in the content of carotenoids and vitamin C. According to the French Pharmacopoeia, the dried leaves of grapevine should contain at least 4% of total polyphenols and 0.2% of anthocyanins [Pharm. Franc. X., 1996; Jonadet 1983; Lardos & Kreuter 2000; Laparra et al. 1989].

The leaves of the red varieties are very rich in tannins from the catechin group. The composition in tannins of leaves depends on the phase of development and on their position on the plant. In autumn, catechin, gallocatechin and epicatechingallate can be found in the leaves. From catechins and/or leukoanthocyanidines so called oligomeric proanthocyanidins, colourless substances, are formed. The greatest part of anthocyans consists of malvidin glucosides but also delphinidin, cyanidin and petunidin glucosides occur [Laparra et al. 1989]. The highest content of anthocyans can be detected in the red leaf especially in autumn, in the time between the vintage and the shedding of leaves [Raynaud, 2006,

Samsung Bioepis Exhibit No. 1002
Page 1106

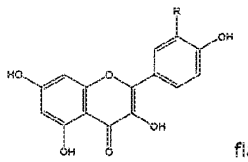
Darné & Glories 1988]. From a pharmacological point of view, the polyphenols, for example flavonoids, are the most important substance group [Bruneton 1999; 2002; Raynaud, 2005; Diaz Lanza et al. 1995].

Analytically the following chemical compounds have been determined in the leaves of *Vitis vinifera*:
- *Flavonoids* (up to 3.5% for red vine leaf, the content is higher in green leaves 4-5%): including kampferol-3-0-glucosides, quercetin-3-O-glucosides
- *Tannins*: procyanidolic oligomers (proanthocyanidins, about 4%) including constituents monomers of cathechin epicatechin
- *Stilbenes*: resveratrol and viniferins [Chung et al. 2003]
- *Fruit acids*: including tartaric acid, malic acid, succinic acid, citric acid, oxalic acid
- *Phenylacrylic acid derivatives*: p-coumaroyl acid, caffeoyl acid, feruloylsuccinic acid

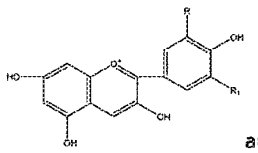
In a recent comparative study of 135 samples of grapevine leaves of different origin, the flavonol, anthocyanin and polyphenol contents have been determined. Total flavonol content was found to be between 0.6% and 3.5%, anthocyanin content between 0.2% and 1.45% and polyphenol content between 4.6% and 18.9%. HPLC methods used for determining anthocyanins and flavonols in grapevine leaves were validated and findings were compared to results produced by assays described in the French Pharmacopoeia. Whereas the correlation between conventional photometric and HPLC methods was satisfactory for anthocyanins, the correlation between the pharmacopoeia assay for total polyphenols and the HPLC analysis for flavonols was poor. As flavonol compounds are considered relevant for the vasoprotective effect of grapevine leaves, their content in starting material used in the production of herbal medicines needs to be quantified [Schneider et al. 2008].

Overview on main polyphenolic compounds in Grape Vine

Flavonoids	Flavones (Quercetin Kaempferol), Flavanes
Anthocyans	Leucoanthocyanidins, Anthocyanidins -responsible for the blue and red coloring of leaves, flowers and fruits -The concentration of anthocyans in the red colored leaf is high.
Catechins	• Grapevine leaf is rich in cathechins • Concentration of cathechins is dependent on: ->the leaf's position on the plant the phase of development of the leaf • In autumn cathechin, gallocathechin, epicathechingallat are present in the leaf.
Stilbenes	Resveratrol, *trans*-Resveratrol belong to the stilbenes (phenolic sub group) is a phytoalexin= stress-induced plant metabolite resveratrol can be found only in stressed leaves stress factors : i.e. fungal infection, UV-irritation, injury ->resveratrol is present in different forms depending on the stage of the plant's stress answer

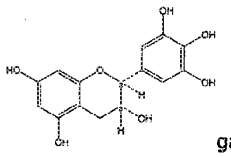


flavonol

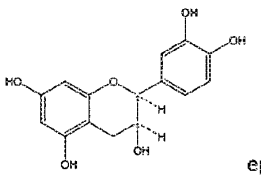


anthocyan

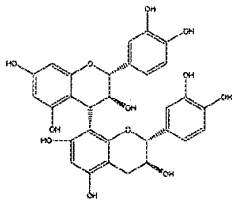
Samsung Bioepis Exhibit No. 1002
Page 1107



gallocatechin



epicatechin



procyanidol

Primary pharmacodynamics

In Europe, the leaves of *Vitis vinifera* are documented in the literature of traditional medicine through several comprehensive reviews for their astringent and homeostatic properties where they are utilized in the treatment of diarrhoea, bleeding, haemorrhoids, varicose veins and other circulatory and venous diseases [Bombardelli 1997; Anonymous 2003; 2004; Petrini et al. 2003; Schneider, 2007i; 2007ii; Schneider et al. 2008; Schaefer & Red 2003; Volonté & Petrini 2004; Volonté et al. 2003; Weber, 2000].

Anti-inflammatory and anti-oedematous effect

In an *in vitro* study, venular endothelial cells were isolated from Wistar rats and cultivated on porous filters to confluent monolayers. These preparations respond to certain release products from simultaneously activated blood platelets and polymorphonuclear granulocytes (PMN) with a rise in hydraulic conductivity that, *in-situ*, would lead rapidly to local oedema, arteriolar constriction and venular thrombosis. In this model, selectively activated PMN alone induced only a modest increase in endothelial hydraulic conductivity that could be prevented by uric acid, an antioxidant. ASA prevented the activation of the blood cells. A standardized extract from grapevine leaves (AS 195), containing in particular the flavonoids quercetin-3-O-ß-D-glucuronide and isoquercitrin (quercetin-3O-ß-D-glucoside), not only prevented the deleterious effect of the release products on the venular endothelial monolayers but, applied promptly to an endothehium damaged by prior exposure to these release products, resulted in the repair of the endothehium [Nees et al. 2003]. In another study, the scavenge by procyanidines (polyphenol oligomers from *Vitis vinifera* seeds CAS 85594-37-2) of reactive oxygen species (ROS) involved in the onset and the maintenance af microvascular injury has been studied in phosphatidylcholine liposomes (PCL) using two different models of free radical generation. In an iron-promoted (Fenton-driven) model, procyanidines had a remarkable, dose-dependent antilipoperoxidant activity (IC50 = 2.5 µmol/l), more than one order of magnitude greater than that of the monomeric unit catechin (IC50 = 50 µmol/l). In the second model, procyanidines were highly effective in preventing conjugated diene formation in both the induction (IC50 = 0.1 µmol/l) and propagation (IC50 = 0.05 µmol/l) phases. The scavenging effect of tocopherol was weaker with IC50 of 1.5 and 1.25 µmol/l [Maffei Facino et al. 1994; Wollina et al. 2006].

The grapevine leaf extract is characterized by its content in flavonol glycosides and glucuronides, in particular quercetin-3-O-β-D-glucuronide, isoquercitrin (quercetin-3-O-β-glucoside), and kaempferol-3-O-glucoside. The most informative investigations were performed using confluent venular endothelial cells from animal and from human origin [Nees, 2003]. An attack by release products from simultaneously activated blood platelets (P) and polymorphonuclear granulocytes (PMN) leads to a breakdown of the venular endothelial barrier. Clinically this would result in formation of oedema and

Samsung Bioepis Exhibit No. 1002
Page 1108

constriction of microvessels. Red vine leaf RVLE extract was able to support the repair of the venular barrier after an attack by said release products. These effects could be demonstrated on cells of from animal origin as well as on cells isolated from the human heart [Nees 2003i, 2003ii]. Quercetin-3-O-ß-D-glucuronide that had been isolated from the extract and was shown to be the major metabolite being present in human plasma after ingestion of RVLE acted in the same way. The extent to which cellular gaps opened and allowed the supernatant to flow through could be measured as "hydraulic conductivity". 0.7 mg dry RVLE/ml incubation medium nearly prevented the opening of the barrier in the presence of activated PMN/P. Preincubation of the venular cell layer with 50 µM quercetin-3-O-ß-D-glucuronide for 7 days markedly reduced the hydraulic conductivity compared to untreated cells.

In a publication by [Jonadet et al. 1983], the authors describe studies conducted with anthocyanosides extracted from *Vitis vinifera* (a), *Vaccinium myrtillus* (b) and *Pinus maritimus* (c). The results obtained *in vitro* indicated that these compounds inhibit elastase, a proteolytic enzyme which plays a role in the deterioration of conjunctive tissue and elastic fibers and is involved in certain pathological vascular conditions. The IC50 values are 0.13 mg/ml for (a), 0.20 mg/ml for (b) and 0.31 for (c). Lineweaver-Burk curves revealed that the inhibition was not competitive. Results obtained *in vivo* show that the angioprotective activities of these compounds can be classified in decreasing order as follows: (a), (b) and (c).
Nees developed a measurable and reproducible *in vitro* experimental model to investigate the effect of substances capable of modifying the hydraulic conductivity of the endothelial barrier of the venules. In *in vitro* experiments, this extract has been shown to have a "sealing" effect on the endothelium of the venules and a protective action against fluid extravasation induced by incubation with chemical mediators of inflammation [Nees S et al. 2003i].
Red vine leaf extract (RVLE) prevented the deleterious effect of the release products of P and PMN on venular endothelial cells. In addition, the extract was able to support the repair of the venular barrier after an attack by said release products [Smith, 1999].
The anti-inflammatory activity of the oligomeric stilbene a-viniferin from *Vitis* has been also tested as well as its mode of action though inhibition of cyclooxygenase-2 and inducible nitric oxide synthase [Chung et al. 2003].

Hepatoprotective activity

The hepatoprotective effect of ethanolic extract and its four different fractions ($CHCl_3$, EtOAc, n-BuOH, and remaining water fraction) of *Vitis vinifera* L. leaves was investigated against carbon tetrachloride (CCl_4)-induced acute hepatotoxicity in rats. The ethanolic extract was found active at 125 mg/kg dose (per os). The ethanolic extract was fractionated through successive solvent-solvent extractions and the n-BuOH fraction in 83 mg/kg dose possessed remarkable antioxidant and hepatoprotective activities. Liver damage was assessed by using biochemical parameters (plasma and liver tissue MDA [malondialdehyde], transaminase enzyme levels in plasma [AST-aspartate transaminase, ALT-alanine transferase] and liver GSH [glutathione] levels). Additionally, the pathological changes in liver were evaluated by histopathological studies. Legalon 70® Protect was used AS standard natural originated drug [Orhan et al. 2007].

Antimicrobial activity

Ethanol extracts of *Vitis vinifera* (leaves, raw fruits, young branches; 2:1:1, v/v/v), were investigated for their antimicrobial activity against 14 pathogenic bacterial species and a yeast, *Candida albicans*, using the agar well diffusion method, 19 Turkish traditionally used medicinal plants. *Vitis* leaves showed broad-spectrum antimicrobial activity [Oskay & Sari 2007].

Samsung Bioepis Exhibit No. 1002
Page 1109

Antioxidative activity

The effect of grape leaf extract (GLEt) on antioxidant and lipid peroxidation states in liver and kidney alcohol induced toxicity has been evaluated. *In vitro* studies with DPPH* and ABTS* (cation radical) showed that GLEt possesses antioxidant activity. *In vivo* administration of ethanol (7.9 g/kg bw/day) for 45 days resulted an activity of liver marker enzymes (AST, ALT, ALP and GGT), lipid peroxidation markers (TBARS, lipid hydroperoxides) in liver and kidney with significantly lower activity of SOD, CAT, GPx, GST and non-enzymatic antioxidants (vitamin E, vitamin C and GSH) in liver and kidney AS compared with control rats. Administration of ethanol along with GLEt significantly decreased the activities of liver markers enzyme in serum towards near normal level. GLEt at a dose of 100 mg/kg was highly effective than 25 and 50 mg/kg body weight. In addition GLEt also significantly reduced the levels of lipid peroxidation and addition, significantly restored the enzymic and non-enzymatic antioxidants level in liver and kidney of alcohol administration rats. This observation was supplemented by histopathological examination in liver and kidney. These data suggest that GLEt exerts its protective effect by decreased the lipid peroxidation and improving antioxidants status, thus proving itself AS an effective antioxidant in alcohol induced oxidative damage in rats [Pari & Suresh 2008].
The antioxidative activity of the ethanolic extract of *Vitis vinifera* L. leaves was investigated. The ethanolic extract of *V. vinifera* leaves at 250 mg/kg dose was found to have the highest antioxidant activity [Sendogdu et al. 2006]. Comparable results have been published by [Kosar et al. 2007], from extracts from fresh, dried and brined leaves of *Vitis* [Monagas M et al. 2006].

Bronchodilatory activity

It has been investigated the effect of grape leaf hydroalcoholic extract on isolated rat tracheal contractions induced by KCl and acetylcholine. The trachea was removed from male adult Sprague-Dawley rat and placed in an organ bath containing Krebs-Henseleit solution and contractions were recorded isometrically. The results demonstrate that the grape leaf extract at 0.5, 1, 2, 4 and 8 mg/ml significantly reduces the tracheal contractions induced by KCl (60 mM) dose-dependently ($P<0.0001$). Acetylcholine (55 µM)-induced tracheal contractions were also attenuated at the same concentration of the extract ($P<0.0001$). The grape leaf extract induced relaxation in the KCl-induced contraction in trachea was unaffected neither by nitric oxide (NO) synthase inhibitor (L-NAME, 100 µM) nor by beta-adrenoceptor antagonist (propranolol 1 µM). Our results suggest that the bronchodilatory effect of grape leaf extract is mediated via the voltage dependent calcium channels on the smooth muscle cells membrane. Furthermore, the beta-adrenergic and NO are not involved. The extract was prepared from dried grape leaves 50 g were wixed with 230 ml of ethanol 70% for 72 hours at room temperature and stirred four times a day. The mixture was filtered and the solvent evaporated. The obtained extract was 9.5 g [Gharib Naseri & Heidari, 2006].

Vasorelaxant effect on isolated rat aorta

The relaxant effect of *Vitis vinifera* leaf hydroalcoholic extract (VLHE) on isolated rat thoracic aorta contractions induced by phenylephrine and KCl, and the role of aorta endothelium on this action has been investigated. Rat aorta was removed and placed in an organ bath containing Krebs-Henseleit solution and aorta contractions were recorded isometrically. The results demonstrate that the VLHE at 0.125, 0.25, 0.5, 1 and 2 mg/ml reduces the endothelial intact aorta contracted by phenylephrine (1 mu M) significantly and dose-dependently. Endothelial denuded aorta showed the same relaxation but in much less extent. The IC_{50} of these two groups were 0.454±0.08 and 1.73±0.23 mg/ml respectively. VLHE also reduced the aorta contractions induced by KCl (80mM). The relaxatory effect of VHLE on KCl-induced contractions were less than those evoked by phenylephrine. Soluble guanylate cyclase inhibitor (methylene blue, 10 mu M) and nitric oxide synthase inhibitor (L-NAME, 100 mu M) reduced the VHLE-induced relaxation in the intact aorta significantly but, atropine (1 mu M) was unable to decrease this vasorelaxant effect. These results suggest that the most vasorelaxant effect of VHLE

Samsung Bioepis Exhibit No. 1002
Page 1110

on rat aorta is endothelium-dependent and also nitric oxide (NO) and cGMP are involved in this action [Gharib Naseri et al. 2004].

Spasmolytic effect

The effect of grape leaf hydroalcoholic extract (GLHE) on rat colon contractions induced by some spasmogens has been investigated. A piece of distal colon from male adult Wistar rats were dissected and mounted in an organ bath containing Tyrode solution and colon contractions recorded by an isotonic transducer under 1g resting tension. The GLHE (0.5- 4 mg/ml) reduced the contractions induced by KCl (60 mM), $BaCl_2$ (4 mM), acetylcholine (1 mu M) dose-dependently ($P<0.001$). The spasmolytic effect of GLHE on ACh-induced contraction was unaffected by propranolol (1 mu M), phentolamine (1 mu M), L-NAME (300 mu M), and naloxone (1 mu M). In Ca^{2+}-free but rich in KCl (120 mM) Tyrode solution, cumulative concentrations of $CaCl_2$ induced colon contractions which, were inhibited by the extract. Glibenclamide (3 mu M) had no effect on the extract spasmolytic activity, but tetraethylammonium (5 mM) contracted the pre-relaxed colon induced by the extract. Results suggest that the grape leaf hydroalcoholic extract spasmolytic effect is due to the blockade of the voltage dependent calcium channels and activation of Ca^{2+}-operated potassium channels [Gharib Naseri et al. 2006].
The effect of *Vitis vinifera* leaf hydroalcoholic extract (VLHE) on isolated rat tracheal contractions induced by KCl and acetylcholine has been also studied. The trachea was removed from male adult Sprague Dalwey rat and placed in an organ bath containing Krebs-Henseleit solution. The tracheal contractions were recorded isometrically under 1.5 g initial tension.

Results: The results demonstrate that the VLHE at 0.5, 1, 2, 4 and 8 mg/ml reduces the tracheal contractions induced by KCl (60 mM) significantly and dose-dependently ($P<0.0001$). Acetylchline (55 mu M)- induced tracheal contractions were also attenuated by the same extract doses significantly ($P<0.0001$). The VHLE-induced relaxation in the KCl-induced contraction in trachea was not affected neither by nitric oxide synthase inhibitor (L-NAME, 100 mu M) or beta-adrenoceptor antagonist (propranolol 1 mu M) and by muscarinic receptors antagonist (atropine 30 mu M).

Conclusion: These results suggest that the relaxant effect of VHLE on rat trachea is evoked via voltage dependent calcium channel blockage and beta-adrenoceptors, NO and cholinergic receptors are not involved in this relaxant effect of VHLE [Gharib Naseri & Heidari, 2006].

Diuretic activity

Aqueous and alcoholic extracts of *Vitis vinifera* leaves were tested for diuretic activity in rats. The parameters studied on individual rat were body weight before and after test period, total urine volume, urine concentration of Na^+, K^+ and Cl^-. In the present study alcoholic and aqueous extracts of *Vitis vinifera* leaves (100 mg/kg of body weight) showed increase in urine volume, cation and anion excretion. Furosemide was used as a reference diuretic [Shastry et al. 2002].

Secondary pharmacodynamics

Flavonoid-containing herbal preparations and isolated flavonoids have been reported to exhibit a wide range of biological effects, including antioxidant and enzyme-modulating actions and anti-allergic, anti-atherosclerotic, antithrombotic, antiviral, antibacterial, anti-inflammatory, antiproliferative, anticarcinogenic, antispasmodic and diuretic effects [Middleton et al. 1992, 1996; Hertog et al. 1996; Hollmann and Katan 1999; Pietta 2000].

In vitro tests
In the context with Chronic venous insufficiency (CVI) it would be of interest to evaluate whether grapevine leaf extract exhibits any effects on platelet aggregation. The effect of flavonoids on platelet aggregation was studied *in vitro* using platelet-rich plasma from four healthy male volunteers aged 24,

Samsung Bioepis Exhibit No. 1002
Page 1111

29, 35 and 47 years. The flavonol glycoside quercetin 3-O-β-D-glucoside and various flavonoid aglycones including quercetin, apigenin and (+)-catechin were added to both platelet-rich plasma and washed platelets at concentrations of 0, 0.25, 2.5, 25, 250 and 2500 µmol/l. Indomethacin was used as a positive control. The results showed that the flavonoid aglycones inhibited platelet aggregation but that quercetin-3-O-β-D-glucoside did not significantly affect aggregation at any of the concentrations tested [Janssen et al. 1998].

In vivo tests

A randomised, placebo-controlled, multiple crossover study was conducted, in which 18 healthy volunteers (9 men and 9 women; mean age 25 ± 8 years; BMI 22 ± 1 kg/m^2) were given a preparation of onions equivalent to 114 ± 3 mg quercetin every day for 2 weeks in accordance with a fixed schedule. Blood samples were taken from the participants at defined intervals and the relationship between platelet aggregation and flavonoid concentration was plotted. The results showed that a daily dose of 114 mg quercetin did not have any significant effect on platelet aggregation. The mean plasma concentration of quercetin was 447 ± 117 ng/ml during the study period [Janssen et al. 1998].
Besides the flavonoids, grapevine leaves contain the following types of compound that are likely to be carried over to an aqueous extract [Bombardelli & Morrazoni 1995]:

- anthocyanins
- phenolic acids
- catechins and proanthocyanidins (flavanols)
- anorganic acids, sugars and mineral salts

The anthocyanins present in grapevine leaves are mainly glucosides of cyanidine, peonidine and malvidine [Boucheny A, Brum-Bousquet 1990]. Anthocyanins have been reported to possess antioxidant and vasoprotective properties. There are some doubts whether these effects measured in *in vitro* experiments or in animal studies using high doses are relevant for humans [Prior, 2004]. Absorption of anthocyanins appears to be low [Lapidot et al. 1998]. The amount of anthocyanins administered with 360 or 720 mg grapevine leaf extract is considered negligible in respect of the pharmacodynamic action of the substance.
The phenolic acids present in grapevine leaves are mainly derivatives of cinnamic acid, vanillic acid and caffeic acid [Boucheny A, Brum-Bousquet 1990]. These compounds are ubiquitous in vegetables and preparations of herbal origin and therefore not expected to contribute to the specific pharmacological effects of grapevine leaf extract.
The catechins found in grapevine leaves are mainly catechol and epicatechol [Boucheny A, Brum-Bousquet 1990]. Of the condensed catechins (proanthocyanidins) procyanidins B1 and B2 are the prevailing compounds, followed by procyanidins B3 and B4. Procyanidins have been reported to possess antioxidant and anti-oedematous activity [Bombardelli & Morrazoni 1995]. The study did not investigate the form in which the flavonoids were resent prior to administration. Onions are known to be rich in quercetin glucosides, especially quercetin-4´-glucoside and quercetin-3, 4´-O-diglucoside. After administration of fried onions quercetin glucuronides can be detected in plasma [Aziz et al. 1998; Graefe et al. 2001]. It can be supposed that quercetin glucuronides do not influence platelet aggregation *in vivo*.

Hypocholesterolemic effect

Oral administration of procyanidins from grape seed produced a hypocholesterolemic effect in a high-cholesterol animal feed model (rats). Specifically it prevented an increase in total and LDL plasma cholesterol and a decrease in HDL [Tebib, 1994].
In normal and hypercholesterolemic rabbit aortas, OPCs significantly lowered the amounts of cholesterol bound to aortic elastin compared to controls [Wegrowski, 1984].

Samsung Bioepis Exhibit No. 1002
Page 1112

Antiproliferative activity

Procyanidin-rich fractions from grape *Vitis vinifera* extract showing different mean degrees of polymerization, percentage of galloylation (percentage of gallate esters) and reactive oxygen species-scavenging capacity were tested on HT29 human colon cancer cells. It has been observed that the most efficient fractions in inhibiting cell proliferation, arresting the cell cycle in G_2 phase and inducing apoptosis were the grape fractions with the highest percentage of galloylation and mean degree of polymerization. Additionally, the antiproliferative effects of grape fractions were consistent with their oxygen radical-scavenging capacity and their ability to trigger DNA condensation-fragmentation [Lizarraga et al. 2007].

Antidiabetic activity

The acute and subacute antidiabetic activities of the ethanolic extract of *Vitis vinifera* L., leaves were investigated. The acute effect was studied on the normoglycaemic, glucose hyperglycaemic and streptozotocin-induced diabetic rats; and the subacute effect was studied on same diabetic rats for 15 days. The blood glucose levels were measured by using blood glucose measuring strips based on glucose-oxidase method. The ethanolic extract of *V. vinifera* leaves at 250 mg/kg dose was found to possess a high antidiabetic activity. Mainly condensed tannins and flavonoids were suggested to contribute in the activity [Sendogdu et al. 2006].
The acute and subacute (15 days) hypoglycaemic and antihyperglycaemic effect of the two different doses (250, 500 mg/kg) of the aqueous extract from the leaves of *Vitis vinifera* L. were evaluated in this study. The aqueous extract was further fractionated through successive solvent extractions and the acute effect of different doses of its subfractions, 25 mg/kg for ethylacetate fraction, 80 mg/kg for *n*-butanol fraction and 375 mg/kg for remaining aqueous fraction were investigated using normal, glucose-hyperglycaemic and streptozotocin-induced diabetic rats. Blood glucose levels were measured according to the glucose oxidase method. Tolbutamide was used as a reference drug at a dose of 100 mg/kg. The antioxidant activity of the test samples was studied in the liver, kidney, heart tissues of diabetic rats by measuring malondialdehyde (MDA) and glutathion (GSH) levels. All results were compared to the diabetic control groups. The results showed that the EtOAc fraction was rich in polyphenolics and possessed a significant antihyperglycaemic and antioxidant activity equipotent with the reference hypoglycaemic agent (tolbutamide), when evaluated in diabetic rats [Orhan et al. 2006].

Other activities

The anti-herpes as well as the anti-mutagenic activities from the leaves and procyanidins from *Vitis vinifera* have been also assayed and published in the international literature [Girre et al. 1990; Liviero et al. 1993].

Pharmacodynamic interactions

Pharmacodynamic drug interactions of whole extracts or isolated constituents have not been reported. Various sources in the literature have suggested that the desired therapeutic effect of many flavonoids is usually obtained only after repeated oral intake of the respective preparations. This implies that the flavonoids and their metabolites accumulate in the body, and do not exert the pharmacodynamic effects seen in *in vitro* studies until a pharmacological threshold dose and relevant plasma levels has been reached. This hypothesis was confirmed by the results of a clinical study by [Kiesewetter et al. 2002]. A significant improvement in key CVI symptoms in the active treatment groups versus placebo could not be observed after 6 weeks, but became evident after 12 weeks of treatment. This could also explain why earlier studies reported a "small" or "undetectable" effect following single oral administration.

Samsung Bioepis Exhibit No. 1002
Page 1113

3.1.1. Assessor's overall conclusions on pharmacology

The aqueous and ethanolic extracts of *Vitis viniferae* folium have been used as adjunctive therapy for Chronic venous insufficiency (CVI), and have shown a potential benefit [Abascal & Yarnell 2007]. Based on available preclinical data, it can be concluded that the mechanism of action of orally administered extract in CVI is not well known.

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Data on the pharmacokinetics of the whole extract or relevant components are not available.

Flavonoids are the substances which are regarded as being responsible for the pharmacological properties of grapevine leaf extract. In the plant, flavonoids are generally present in genuine form as glycosides. Grapevine leaf extract as it has been referred previously contains as major flavonoid components quercetin-3-O-glucuronide, isoquercitrin (quercetin-3-O-glucoside) and kaempferol-3-O-glucoside. The substances present in blood after ingestion of plant extracts containing polyphenols usually differ from the native compound, as they are metabolites resulting from digestive and hepatic activity. The examination of pharmacokinetics and metabolism of the major and active constituents of such plant extracts in humans is therefore strongly recommended [Manach et al. 2005].
Various sources in the literature have suggested that the desired therapeutic effect of many flavonoids is usually obtained only after repeated oral intake of the respective preparations. This implies that the flavonoids and their metabolites accumulate in the body and do not exert the pharmacodynamic effects seen in *in vitro* studies until a pharmacological threshold dose and relevant plasma levels has been reached. This hypothesis was confirmed by the results of a clinical study by [Kiesewetter et al. 2000]. A significant improvement in key CVI symptoms in the active treatment groups versus placebo could not be observed after 6 weeks, but became evident after 12 weeks of treatment.

The grapevine leaf extract represents a complex mixture of different classes of compounds, having each their own pharmacokinetic characteristics. A significant portion of the components of grapevine leaf extract belongs to the group of polyphenols, such as anthocyanins, proanthocyanidins, catechins and phenolic acids [Bombardelli & Morazzoni 1995; Boucheny & Brum-Bousquet 1990]. Recently, [Manach et al. 2005] presented a review of various bioavailability studies in humans concerning polyphenols. They came to the conclusion that anthocyanins and proanthocyanidins are absorbed to a very low extent only. As an exception, the major circulating forms of anthocyanins are the intact glycosides [Manach et al. 2004]. The polymeric nature and therefore high molecular weight of proanthocyanidins should limit their absorption through the gut barrier. Hydroxycinnamic acids such as caffeic acid are rapidly absorbed from the small intestine and are conjugated, in particular glucuronidated in the same way as flavonoids. However these compounds are naturally esterified in plant products (as chlorogenic acid) and this impairs their absorption. Only the colonic microflora possesses esterases which are capable of hydrolyzing these bindings. Consequently, the efficacy of absorption of phenolic acids is markedly reduced for their esterified forms compared with the free form [Manach et al. 2004].

3.2.1. Assessor's overall conclusions on pharmacokinetics

No information is available on pharmacokinetic interactions. No information is available on metabolites.

Samsung Bioepis Exhibit No. 1002
Page 1114

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

Single-dose toxicity

The toxicity of vine leaf aqueous extract (4-6:1) following a single oral dose was determined in SPF Wistar rats (3 males and 3 females per group). The doses used were 3000, 4000 and 10.000 mg/kg. There were no signs of toxicity and no mortalities over the 14-day observation period, and no pathological or histological changes were seen at autopsy.

The toxicity of vine leaf aqueous extract (4-6:1) following a single oral dose was also determined in CFI mice (3 males and 3 females per group). The doses used were 2000, 4000 and 10.000 mg/kg. There were no signs of toxicity and no mortalities over the 14-day observation period, and no pathological or histological changes were seen in autopsy [Rabe 2005; ESCOP, 2009].

Repeat-dose toxicity

The toxicity of vine leaf soft extract when given over a 90-day period was determined in SPF Mol-Wistar rats (12 males and 12 females per group) in a study which was subjected to QAU inspection and carried out in accordance with GLP standards. The animals were given oral doses of 25, 125 or 250 mg/kg/day, or a placebo. A satellite group given 250 mg/kg/day was subjected to follow-up observation. No mortalities occurred during the study period. Observations made during the study period on bodyweight, food intake etc. revealed no evidence of any systemic toxic effects due to the extract. The values found during haematological and clinical chemistry testing were within biological limits. The autopsy findings and the results of histological tests did not reveal any evidence of changes due to the extract. The doses of extract used were therefore judged to be non-toxic in the rat.

The maximum daily dose of extract is 720 mg, which represents a dose of 10.3 mg/kg for a person weighing 70 kg. This is about one-thousandth of the maximum dose of extract used in the single-dose toxicity studies. As the maximum dose used in these studies did not give rise to any signs of toxicity, it is extremely unlikely that any toxic effects will occur in man. Preparations containing vine leaf extract have been used in European Union countries for many years and there have been no reports of any harmful effects in man to date. On this basis, it is reasonable to assume that the products reviewed in this report will not pose any safety risk on therapeutic administration [ESCOP, 2009].

Genotoxicity

The mutagenic potential of vine leaf extract was assessed in a range of established *in vitro* and *in vivo* test models (Ames test, point mutation assay and chromosomal aberration assay). It was assessed in an Ames Salmonella/microsome plate assay using Salmonella typhimurium TA-100, TA-1535, TA-98, TA-1537 and TA-1538 with and without metabolic activation; S9 mix prepared from rat liver was used as the activation system. The assay was carried out in accordance with EU recommendations and OECD Guideline 471. Five concentrations of the vine leaf extract ranging from 8 to 5000 µg/plate were used, and positive and negative controls were also included. The results obtained did not provide any evidence to suggest that vine leaf extract has any mutagenic potential [ESCOP, 2009].

Mutagenic potential of grapevine leaf extract was also assessed in two independent point mutation assays carried out on the HGPRT locus of Chinese hamster V79 cells with and without metabolic activation; S9 mix prepared from rat liver was used as the activation system in each case. The assays were designed and conducted in accordance with the guidelines and recommendations on genotoxicity testing that were current at the time. On the basis of preliminary toxicity tests, a concentration range of 3.0 - 5000 µg/ml was selected for use in the assays. Positive EMS and DMBA controls as well as a

Samsung Bioepis Exhibit No. 1002
Page 1115

vehicle control were included. Neither of the assays provided any evidence to suggest that the extract used had any mutagenic potential in the concentration range tested [ESCOP, 2009].

A micronucleus assay was carried out on NMRI mice (5 males and 5 females per group) to establish the potential of vine leaf soft extract for causing chromosomal aberrations. The assay, which complied with the relevant EU and OECD guidelines, was carried out using oral doses of the extract of 1.0, 3.0 and 10.0 ml/kg. Positive controls (cyclophosphamide 40 mg/kg) and vehicle controls (sodium chloride 0.9%) were included. Under the conditions employed, there was no significant increase in micronuclei frequencies in polychromatic erythrocytes.

The methanolic and aqueous extracts from Greek varieties of *Vitis vinifera* demonstrated *in vitro* antimutagenic effects against mutagenicity induced by bleomycin and hydrogen peroxide in Salmonella typhimurium strain TA102 [Stagos et al. 2006].
Oligomericproanthocyanidins from grape seed demonstrated in vitro antimutagenic activity [Liviero et al. 1993].
In addition, among the pharmacologically active flavonoids (quercetin, quercetin 3-O-β-D-glucuronide and isoquercitrin) in the vine leaf extract used is known that quercetin has given positive results in the Ames test. However, the mutagenic potential of quercetin has not been confirmed in *in vivo* tests carried out in rats and mice [Hertog & Hollman 1996].

Carcinogenicity

No data is available. However, it was demonstrated that anthocyanin-rich extracts from bilberry (*Vaccinium myrtillus* L.), chokeberry (*Aronia meloncarpa* E.), and grape *(Vitis vinifera*) inhibited multiple biomarkers of colon cancer in rats [Lala et al. 2006].
There has also been no evidence in the literature so far to indicate that flavonoids as a whole have any carcinogenic properties. Indeed, a large number of *in vitro* studies have been carried out which suggest that flavonoids have anticarcinogenic effects. However, the results obtained *in vitro* have yet to be confirmed in *in vivo* test models. Depending on the type and quantity of food consumed, the human intake of flavonoids is between 100 and 1000 mg per day [Middleton 1996].

In toxicity and carcinogenicity studies carried out on quercetin, promoted by the FDA, four groups of 50 male and female F344 rats were given 40, 400 or 1900 mg/kg of quercetin, or a placebo, in feed over a period of two years. There was no evidence of carcinogenic activity in the female rats and only slight evidence of carcinogenic activity in male rats. The carcinogenic effects, which took the form of renal tubule hyperplasia and nephropathy, were observed only in the high-dose group. No evidence of chronic toxicity was found [NTP: Toxicology and carcinogenesis studies of quercetin (CAS No. 117-39-5) in F344 rats (feedstudies) 1992].

Reproduction toxicity

The maternal and embryofoetal toxicity of an orally administered aqueous preparation of vine leaf soft extract was investigated in pregnant Himalayan rabbits in a study which was carried out in accordance with GLP standards and subjected to QAU inspection. Four groups of animals (12 per group) were used. One group received a placebo whilst the other three groups were given doses of 300, 1000 or 3000 mg/kg/day on days 6-18 of gestation. All doses were administered in a volume of 10 ml/kg. No signs of maternal toxicity were seen during the observation period and there was no visible evidence of foetal retardation. Slight, non-significant skeletal retardations and variations were seen in the foetuses of animals receiving the highest dose (3000 mg/kg of the extract per day). These variations were within biological limits in all cases and did not occur in any of the other groups. There was no evidence of changes due to the extract in any of the other findings, and no teratogenic effects were seen. On the basis of the results obtained, the NOEL for the extract was defined as > 3 g.

Samsung Bioepis Exhibit No. 1002
Page 1116

In the reproduction study conducted with rabbits no evidence of any teratogenic effects was found. The recommended maximum dose is equivalent to a daily intake of 10.3 mg/kg of vine leaf extract. In the Leuschner study up to 3000 mg/kg/day of the extract, were applied without resulting in any teratogenic effect. The recommended maximum human dose of vine leaf extract is about 300 times lower than the highest dose used in the Leuschner study [Leuschner F, Mitterer KE, 1993].
Examination of the influence of *Extractum vitis viniferae* on the pregnant rabbit and the fetus by oral administration [LPT Report No. 7441/92. 1993; ESCOP, 2009].

Daily doses of 3000 mg/kg extract from grapevine leaves administered to female rabbits during organogenesis (6th to 18th day of pregnancy) did not exhibit teratogenic effects [Antistax® SPC, 2005].

3.3.1. Assessor's overall conclusions on toxicology

Several recent data on oral single-dose and repeat-dose toxicity, as well as on genotoxicity and reproduction toxicity of vine leaf have been reported.
In vitro and *in vivo* studies on the mutagenic potential of vine leaf extracts have not shown any evidence of mutagenic activity. Carcinogenic effects are not expected to occur, especially as vine leaf extract has been used in medicinal products for many years without any evidence of such effects being reported.

4. Clinical Data

4.1. Clinical Pharmacology

Pathophysiology of CVI

The term Chronic venous insufficiency (CVI) describes a clinical picture in which chronic venous diseases of diverse aetiology are manifested by similar or related symptoms and complaints. CVI is mostly caused by venous and capillary hypertension, which causes a persistent, chronic, venous stasis in the skin of the lower leg, manifested most characteristically upon postural challenge [Alguire & Mathes 1997]. The impairment of venous return that is responsible for the venous capillary hypertension can be caused by degenerative, dilating venous conditions of the superficial (primary varices), the transfascial (insufficiency of the perforating veins), or the deep system ("deep" varicosis, insufficiency of the main veins) [Jünger et al. 1994]. Chronically disturbed haemodynamics of deep or superficial veins due to obstructed venous segments or valvular incompetence lead usually to trophic changes in the inner ankle area of the lower limbs and disturbances in the microcirculation of the skin have been considered to be major contributors for skin changes associated with chronic venous hypervolaemia and venous hypertension. Cutaneous microangiopathy of clinical relevance such as enlarged, tortuous capillaries surrounded by micro-oedema contributes to the skin alterations in the lower limbs and determines the course of CVI [Fagrell 1995; Jünger et al. 1996]. CVI can be classified according to its haemodynamic, morphological, and clinical aspects. The clinical grading system, based on Widmer's classification, as well as other international classification systems, e.g. CEAP (Clinical Class, Etiology, Anatomy and Pathophysiology), [Widmer LK, 1981; Partsch H, 1994; Porter JM et al. 1995]. The most important therapeutic approaches include compression therapy, physiotherapy, surgical treatment and sclerotherapy.

Drug therapy

Drug therapy is a treatment option in incipient and mild to moderate Chronic venous insufficiency (CVI) (Widmer grades I and II, CEAP Clinical classes 2, 3, or 4a).

Samsung Bioepis Exhibit No. 1002
Page 1117

Topical drug treatment

Antioedema drugs are mostly plant derived substances for which an antiexudative and antioedematous action and efficacy have been confirmed in experimental studies and clinical trials. Experimentally, most of them have a membrane stabilising action that leads to a decrease in capillary permeability. Some of these substances also have venotonic properties.
Long-term treatment with antioedema drugs can also be considered in primary and secondary CVI or if invasive measures are not applicable [Gallenkemper et al. 1998]. The majority of these products contain plant extracts or semisynthetic derivatives of plant-derived substances. The most commonly used are coumarins (α-benzopyrone derivatives); saponins/horse chestnut seeds extracts; flavonoids (γ-benzopyrone derivatives); rutin, diosmin, and hesperidin; anthocyanins and procyanidins.
Procyanidins, like the anthocyanins, are flavonoids that are not available as monosubstances. Procyanidins are present in relevant amounts in standardised extracts such as grapevine leaf extracts (*Vitis vinifera*) and are formed through condensation reactions of flavonols [Hostettmann K et al. 1994].
The complex syndrome of symptoms involved in CVI led to investigate the activity of vine leaf preparations on two distinct parameters affecting CVI. On the one hand the effects of the product on microcirculation, on the other on the typical objective and subjective symptoms of CVI such as the presence of oedema and the typical CVI complaints "tired, heavy, and swollen legs", or tension and pain in the legs.

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

No data available.

4.1.1.1. Assessor's overall conclusions on pharmacodynamics

At present, the mechanism of action of vine leaf water extract in chronic venous insufficiency cannot be considered clarified.

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

The vine leaf extract is a complex extract containing more than 200 different identified substances. The clinical effects of the grapevine leaf extract cannot be attributed to a specific individual constituent but can be ascribed with reasonable plausibility to the extract as a whole. It is therefore virtually impossible to perform classical pharmacokinetic studies with the complete product and pharmacokinetic studies of individual constituents such as flavonoids will only provide limited information with regard to clinical efficacy.

Flavonoids (quercetin glucuronide, kaempferol glucosides) are quantitatively important constituents of the whole extract. The systemic bioavailability of flavonoids is probably relatively low and variable. Orally administered flavonoids are susceptible to pre-systemic metabolism by the intestinal flora. It is likely that the absorbed metabolites formed by the microflora in the gut contribute to the biological action of orally administered flavonoids. Systemic flavonoids are metabolised in the liver primarily by conjugation (methylation or sulfuronidation) and/or glucuronidation. The resulting metabolites are excreted either with the urine or with the bile into the intestine, where they undergo further metabolism. The pharmacokinetic input function of systemic flavonoids may well be further extended by this enterohepatic recycling process [Manach & Donovan 2004].

Samsung Bioepis Exhibit No. 1002
Page 1118

4.1.2.1. Assessor's overall conclusions on pharmacokinetics

Data on pharmacokinetics of vine leaf extract or relevant components are not available in humans. No reliable methods exist to determine simultaneously the plasma levels of all active ingredients present in the whole extract.

4.2. Clinical Efficacy

Several publications referring to human clinical studies with medicinal products containing *Vitis vinifera* were found in the literature.
Three of the published studies investigated the safety and efficacy of a dry extract of grapevine leaves (4-6:1) extraction solvent water (AS 195).
The safety and efficacy specific information of these studies is presented below.

The clinical information available for aqueous extracts of grape vine leaf investigates the activity on two distinct parameters affecting Chronic venous insufficiency (CVI). On the one hand the effects of the product on microcirculation, on the other on the typical objective and subjective symptoms of CVI such as the presence of oedema and the typical CVI complaints "tired, heavy, and swollen legs", or tension and pain in the legs.

Clinical parameters measured

In all clinical studies, changes in leg volume and/or calf and ankle circumference were included in the endpoints studied. All confirmatory studies planned to show efficacy on subjective symptoms of CVI used the lower leg volume determined by water displacement plethysmography as the primary efficacy endpoint. Other endpoints included the lower leg diameter (at calf height and at the ankle), visual analogue scales (VAS: 0-10 cm) for the main symptoms ("tired, heavy legs", "sensation of tension", "prickling sensation", and "pain in the legs"), and the global assessment of efficacy and tolerability by the patient and by the doctor according to a 4-point verbal rating scale (VRS: "good", "satisfactory", "not satisfactory", and "poor"). These measurements are consistent with the guidelines of the German Society for Phlebology.
In the double-blind, placebo-controlled cross-over study with two treatment sequences of 6 weeks each separated by a 4-week placebo washout period, microcirculation in the most affected CVI leg was measured by laser doppler fluxmetry (685 nm, penetration depth approximately 1 mm) and transcutaneous oxygen monitoring.
Patients were usually questioned about their subjective well-being. They were also instructed to spontaneously report any adverse events experienced. Laboratory tests (haematology, clinical chemistry, and urinalysis) and blood pressure and pulse measurements were carried out in most studies to record safety.

4.2.1. Dose response studies

From the results of [Kiesewetter et al. 2000], a single daily dose of 360 mg of grapevine leaf extract is as effective and well tolerated as a daily dose of 720 mg. On average, the higher dose regimen offers greater efficacy in terms both of the extent and duration of action, but is roughly equivalent with regard to subjective symptoms reduction. The lower dose, however, is considered to be sufficient in the majority of patients and has been chosen to reduce the intake of extract, thus most likely decreasing the likelihood of observing adverse events. Consequently, this is the dosage that has been approved in some marketing authorisations.
No pharmacokinetic or pharmacodynamic studies, however, were performed to support the posology and daily dose proposed.

Samsung Bioepis Exhibit No. 1002
Page 1119

4.2.2. Clinical studies (case studies and clinical trials)

Several publications referring to human clinical studies investigating the safety and efficacy of a dry extract of red *Vitis vinifera* leaves (4-6:1) extraction solvent water (AS 195) were found in the literature. Some others studies have been assessed in this AR, that originate from confidential in house files of Boehringer Ingelheim. The company allowed using those data for assessment and monograph/list entry establishment.

Double-blind, Placebo-controlled studies

[Kiesewetter et al. 2000]: In a 12-week, randomized, double-blind, placebo-controlled, parallel-group, multicenter study conducted in accordance with the principles of Good Clinical Practice (GCP), the efficacy and safety of once daily doses of 360 and 720 mg RVLE (AS 195) were compared to placebo in male and female outpatients aged between 21 and 80 years old (with 52% ≥ 60 years) with body weights ranging from 46 to 120 kg (Broca index: median 7.3, range: -30 to +82%; this correspond to a BMI of approx. 25 kg/m^2). And stage I and incipient stage II Chronic Venous Insufficiency (CVI) with no major dystrophy of the skin. The majority (72%) of the patients were women. Compression therapy and the administration of diuretics were not permitted during the study period.
Patients were randomly assigned to a double-blind treatment with placebo, 360 mg RVLE or 720 mg RVLE once daily for 12 weeks, preceded and followed by a single-blind 2-week placebo treatment for baseline run-in and end-of-trial washout, respectively. Study criteria were evaluated at baseline, after 6 and 12 weeks of treatment and 2 weeks after discontinuation of treatment. Of the **260** patients enrolled and randomized, 219 completed the study in accordance with the protocol, 86 test subjects took a single daily dose of 360 mg AS 195, 84 took 720 mg AS 195/day and the remaining 87 test subjects received a placebo. During weeks 13 and 14 none of the participants received any further medication.

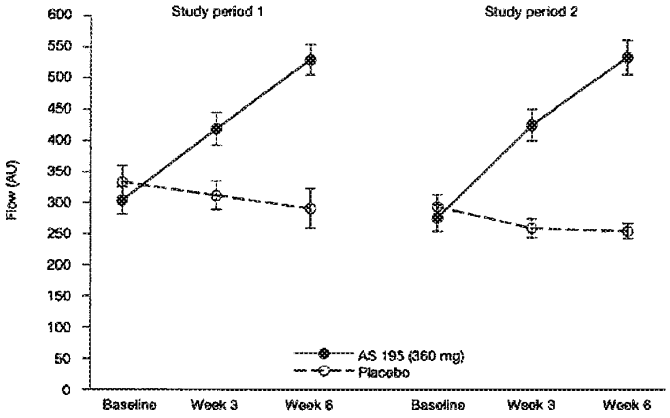
Subjectively, there was an improvement in CVI symptoms at 6 weeks with all treatments, but a further improvement at week 12 was seen only in the active treatment groups: at 12 weeks, the changes compared to baseline were significantly greater ($p < 0.001$) in both active treatment groups than in the placebo group. The study demonstrated that once daily doses of 360 and 720 mg RVLE were effective in the treatment of mild CVI, reducing significantly lower leg edema and improved CVI-related symptoms to a clinically relevant extent.

The treatments were well tolerated. All 260 patients who were enrolled and randomized were included in the safety analysis. This included three patients who withdrew from the study before visit 2 (i.e. before entering the treatment phase). 257 patients received at least one dose of the randomized study medication. Most patients completed the study up to 84 days of treatment. The mean duration of treatment for the 360-mg dose of AS 195 was 81.9 days (n = 86) whilst the mean duration of treatment for the 720-mg dose of AS 195 was 79.7 days (n = 84). Placebo was taken by 87 patients during the randomized treatment period (mean duration of treatment: 79.3 days) and by all patients during the 14-day run-in period (n = 260) and the 14-day follow-up period (n = 246). A total of 34 adverse events (AEs) were reported in 31/260 patients: 3 AEs in 3/260 subjects treated with placebo during the run-in phase; 3 AEs in 3/246 patients treated with placebo during the follow-up period; 10 AEs in 10/87 patients treated with 360 mg AS 195; 2 AEs in 2/85 patients treated with 720 mg AS 195; and 16 AEs in 13/88 patients treated with placebo. Most AEs were considered to be mild (n = 21) or moderate (n = 9). Four AEs were classified as severe, three in patients taking the placebo (phlebitis of the large saphenous vein; knee surgery; abdominal discomfort) and one in a patient taking 360 mg AS 195 (leg hematoma following minor trauma). Six AEs were rated as likely to be causally related to the study medication by the investigator; of these, two (mild constipation and mild hair thinning) were

Samsung Bioepis Exhibit No. 1002
Page 1120

reported in patients treated with 360 mg AS 195, whilst the other four occurred in patients treated with placebo. Gastrointestinal disorders (abdominal discomfort, diarrhea, dyspepsia, dry mouth or retching) were the most frequently cited AEs (n = 11), followed by infections (n = 6), headache (n = 4) and disorders of the musculoskeletal system (n = 4). Two AEs which occurred during treatment with placebo required hospitalization and were labelled as „serious"; there was one incident of vaginal hemorrhage during the run-in phase and one incident of severe acute osteoarthritic complaints in the knee of the affected leg during the randomized treatment period. Three other patients were withdrawn following an AE: 1 patient was withdrawn during the placebo run-in period due to moderate abdominal discomfort; 2 patients were withdrawn from randomized treatment with the placebo, one because of phlebitis of the greater saphenous vein and one because of severe abdominal discomfort. The clinical laboratory data, blood pressure and pulse rate data and 12-lead ECG recordings did not indicate any significant mean or individual changes which were likely to have been due to the study medications. Overall tolerability was rated as follows for the 3 treatments: 360 mg AS 195: good 81%, satisfactory 19%; 720 mg AS 195: good 94%, satisfactory 6%; placebo: good 68%, satisfactory 30%, not satisfactory 2%. The study was conducted in multicentric settings, but most measurements were carried out by only two investigators, thus this trial can be considered basically a monocentric one

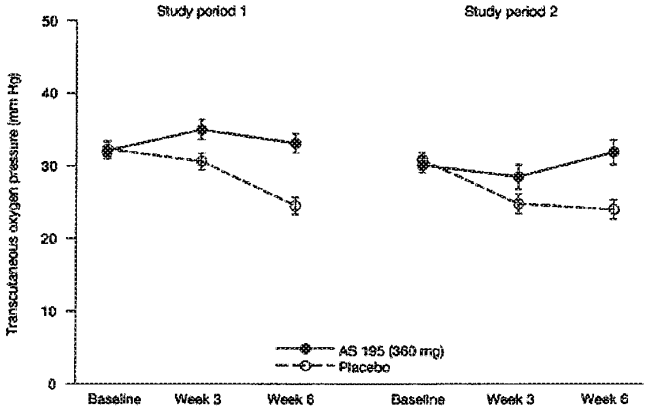
[Kalus et al. 2004]: The effect of AS 195 on cutaneous microvascular blood flow, transcutaneous oxygen pressure (tcpO2), and leg oedema was investigated in a randomised, double-blind, placebo-controlled, crossover study in male and female patients, aged ≥ 18 years with an average age of 66 years, with confirmed CVI stage I or II. In this efficacy study, **71** patients (70 during the second phase) were treated with either placebo or 360 mg AS 195 film coated tablets. The first group (n=36) received AS 195 360mg once daily during a first 6-week treatment period, followed by a 4-week placebo washout period and then placebo during the second 6week treatment period. The second group (n = 35) started with placebo and received AS 195 360mg after the placebo wash-out. After 6 weeks, patients in the AS 195 group had increased microvascular blood flow values and transcutaneous oxygen pressure (tcpO2). The active substance group showed a statistically significant increase of 241.8±18.7 Arbitrary Units (AU), while a reduction of 41.0±18.7 AU was recorded in the placebo group ($p<0.0001$).



Microcirculation, measured in arbitrary units (AU) (laser Doppler flow measurement at Doppler frequencies of 10.1–37.2 kHz, mean ± SEM, after 10 minutes of standing) (from [Kalus et al. 2004])

Samsung Bioepis Exhibit No. 1002
Page 1121

The $TcpO_2$ oxygen reading also rose significantly from baseline levels in the AS 195 group, by 1.35±0.97 mmHg, contrasting with a reduction in the placebo group of 7.27±0.97 mmHg.



Transcutaneous oxygen pressure (mean ± SEM, after 10 minutes of standing) (from [Kalus et al. 2004])

These changes in the measured parameters were associated with corresponding volume changes in the lower leg. After just three weeks of treatment, statistically significant differences were also observed in ankle and calf circumference. After six weeks, these circumferences had fallen by 0.39 and 0.54 cm respectively, in the active group, compared to increases in the placebo group of 0.29 and 0.14 cm respectively. The authors of the study concluded that the administration of AS 195 could have a positive effect on the course of CVI. The minimum number of days of exposure was 38 in the AS 195 and 24 in the placebo group, the maximum number 46 in the AS 195 and 45 in the placebo group. Thirteen (18.3%) subjects out of 71 experienced 16 adverse events (AEs) during the 16 week trial. Ten AEs (14.1%) were of mild, 4 (5.6%) of moderate and 1 (1.4%) of severe intensity. None of the AEs were assessed as related to the trial medication. One patient died from a cardiac arrest. He had been treated with placebo and never received AS 195 in this trial. All patients assessed the overall tolerability as good or satisfactory. Laboratory parameters did not change during the study [Kalus et al. 2004].

[Limoni C, 1996]: A four-week, multicentre, randomised, double-blind versus placebo, parallel-group safety trial to evaluate the tolerability profile of certain capsules (water extr. *Vitis vinifera* siccum 4-6:1) in male and female patients suffering from chronic venous insufficiency. The study population consisted of **105** patients, male and female, aged between 20 and 70 years with chronic venous insufficiency of grade I, II, and III according to Widmer. The patients received 3 capsules of that extract or placebo per day during 28 days. Seventy-two patients were randomised into the active treatment group and 33 into the placebo treatment group. In the absence of a strictly diagnosed CVI, patients with a subjective feeling of heavy and tired legs were admitted as well. Primary endpoints were the incidence of adverse events and the rating of the overall tolerability. Secondary endpoints were laboratory tests and physical examination. There were no differences in tolerability between the two treatment groups. The proportion of volunteers reporting adverse events was similar in both treatment groups: 40.2% in the active treatment group and 36.4% in the placebo group. The most frequent adverse events were mild gastrointestinal disorders, sleep disturbances, and tiredness. Laboratory values did not change in either treatment group during the trial period. The capsules were well tolerated by 84.9% of the volunteers. No severe adverse events were reported.

Samsung Bioepis Exhibit No. 1002
Page 1122

[Vix et al. 2007]: This 12-week, double-blind, randomised, placebo-controlled, multicentre trial was carried out to evaluate the efficacy and tolerability of film-coated tablets, extr. *Vitis vinifera* (***4-6:1 water***) 360 mg/day orally, in male and female patients suffering from chronic venous insufficiency. The design and methodology used closely reflected those used in BI trial by Schaefer 2004).
The time course of the change from baseline in limb volume was similar for both treatment groups with a more marked improvement of leg oedema over time in patients treated with these tablets. After 84 days of treatment, limb volume was reduced by -21.38 (±11.30) g of displaced water in the verum group. The adjusted mean reduction in limb volume in the placebo group was less pronounced with -10.71 (±11.56) g. However, the difference between treatment groups (-10.67 ±14.35 g) was not statistically significant.

For the secondary endpoint "change from baseline in calf circumference", the adjusted mean difference to placebo after 42 days of treatment with these tablets was -0.42±0.22 cm in the FAS (p=0.0596) vs. -0.31±0.24 cm on study day 84 (p=0.1851). In the Per-Protocol Set (PPS), the difference to placebo was -0.47 (±0.23) cm on study day 42, with a p-value just reaching significance at 0.0445. The evaluation of the four subjective symptoms of CVI, as assessed by VAS, yielded similar results: after 42 days of treatment, the adjusted mean differences between active treatment and placebo were -0.44 cm for "tired, heavy legs", -0.73 cm for "sensation of tension in the legs", and -0.48 cm for "pain in the legs" (vs. -0.38 cm, -0.04 cm, and 0.18 cm on study day 84). There was no difference between treatment groups regarding the symptom "tingling sensation in the legs". Global efficacy was rated as good or satisfactory by 70.9% of all patients treated with these tablets and 67.7% of all patients allocated to placebo whereas the investigators' ratings were 69.0% and 62.6%, respectively. The assessment of quality of life by the Tuebingen QoL questionnaire resulted in a better mean score for verum group compared with placebo regarding the subscale "leg complaints", the subscale most specifically related to CVI. The differences, however, were not statistically significant.

[Schaefer & Petrini 2004]: This placebo controlled, randomised, parallel group study was carried out in 2004. **247** CVI patients (CEAP-Scores 3 or 4a) were entered in the study: 121 were treated with the grapevine leaf extract AS 195 360 mg as a single tablet and 126 with placebo for 12 weeks. For the primary endpoint, the difference related to placebo, adjusted for centre effect and baseline values, in changes of displaced water from baseline until Day 84 was -10.42 cm (SE: 11.84; 95% CI: -33.76, 12.92). The difference in changes between the two treatment groups was not statistically significant. Subjective CVI symptoms diminished in both treatment arms similarly during the first three weeks. A statistically significant difference between the two treatment groups in favour of verum, was found for the symptoms "sensation of tension in the legs" at Day 42 (p=0.0211, ANCOVA) and "pain in the legs " at Day 84 (p=0.0222, ANCOVA). At all other time points no statistically significant difference was detected between the two treatment regimens for the symptoms "tired heavy legs" as well as "tingling sensation" in the legs. After 84 days of treatment, the limb volume as measured by the water displacement method was statistically not significantly more reduced in subjects allocated to verum than in those randomised to placebo. Careful inspection of the results showed that the unexpected high intra- as well as inter-individual variability of limb volume determinations contributed significantly to the outcome of the study. The variability between 2 or 3 replicates of the limb volume determination, for example, exceeded 20 g of displaced water in more than 20% of patients (range 0% to 44.1%) in 8 out of 23 study sites (35%). This study was negative in terms of a primary endpoint, although there was evidence of symptomatic benefit from the active treatment.

[Schaefer E & Petrini LE, 2003]: A 17 week, randomised, double-blind, placebo controlled cross-over trial to evaluate the efficacy of water extr. *Vitis vinifera* ***4-6:1*** film coated tablets (AS 195) given as 2 capsules of 180 mg of AS 195 daily 360 mg/day p.o., in **179** male and female Japanese patients with swellings of calf and ankle due to disorders of venous reflux belonging to Class 1 according to Porter's classification, in improving microcirculation of the skin in the leg of male and female patients

Samsung Bioepis Exhibit No. 1002
Page 1123

suffering from chronic venous insufficiency The microcirculation study has demonstrated that objective signs associated with CVI (microvascular blood flow, $tcpO_2$, ankle circumference and calf circumference) can be improved significantly after just 3 weeks of oral treatment with a single daily dose of 360mg of the grapevine leaf extract AS 195. These results build on those from Harrison trial (1998) showed that AS 195 reduces lower leg oedema and calf circumference in patients suffering from CVI treated once daily for 12 weeks. Out of the 179 evaluable subjects 38.5% experienced a marked and 42.5% a moderate global improvement of CVI symptoms. Improvement was rated as "moderate" by 59.8% of the patients with regards to heaviness/tiredness, 69.9% with regards to tension, 79.2% to tingling, 74.0% to pain, and 74.4% to itching. Circumference of calf (mean±SD) was 33.5±2.99 cm prior to treatment and 33.0±2.94 cm at 12 weeks, of the ankle (mean±SD) 22.3±1.84 cm prior to treatment and 21.9±1.82 cm at 12 weeks after treatment ($p<0.05$). These differences were not statistically significantly different from baseline.

Open-label, Observational studies

[Schaefer et al. 2003]: The 6-week, open, uncontrolled multicentre safety trial was conducted to specifically evaluate the tolerability and safety of AS195 film-coated tablets, 360 mg/day *per os*, in **65** male and female patients, aged 25 - 82 years, suffering from chronic venous insufficiency grade I or II (Widmer classification). The verum group received two film-coated tablets once daily for 42 days (360 mg/day). The primary objective of the study was to assess the tolerability and safety of AS 195 extr. *Vitis vinifera* siccum ***4-6:1***) film-coated tablets. The observational study was conducted in accordance with the requirements of the Swiss licensing authority (Swissmedic: application document) in a total of 11 study centres (general practitioners) between June and October 2002. Three visits (study enrolment, baseline examination at the start of the study, final examination at the end of the study, with a follow-up examination if necessary) were planned. The study was carried out in accordance with GCP. At the end of the study, all subjective symptoms of CVI (tired, heavy legs, sensation of tension in the legs, tingling sensations in the legs, pain in the legs) were statistically significantly improved. The global assessment of efficacy by the patients and by the investigators was rated as good or satisfactory in most of the patients. The primary variable was the number and intensity of adverse events (AEs), with particular emphasis placed on AEs regarded by the investigator to be treatment-related. Secondary safety variables included the global assessment of tolerability by both the patients and investigators as well as vital sign assessments. AS 195 film coated tablets were well tolerated during the whole study. Overall, six patients (9.2%) experienced a total of 7 AEs of mild or moderate intensity judged by the investigator as potentially causally related to study medication. They included gastrointestinal problems (4 patients), headache (1 patient), anorexia (1 patient), and erythematous rash (1 patient). Four patients of whom experienced a significant AE necessitated study discontinuation in three cases and dose reduction in one case. Most patients rated the global tolerability of AS 195 film-coated tablets as good (49 patients, 75.4%) or satisfactory (12 patients, 18.5%). Three patients (4.6%) rated tolerability as bad. One patient (1.5%) gave no rating. The global assessment of tolerability by the investigators was in good agreement with those of the patients. The investigators rated the tolerability as good in 50 patients (76.9%), as satisfactory in 10 patients (15.4%), as not satisfactory in one patient (1.5%), and as bad in two patients (3.1%). No relevant changes of vital signs (systolic and diastolic blood pressure, pulse rate) were measured. Patient´s compliance was rated as good [Schaefer et al. 2003].

Assessment The present open-label, multicentre observational study demonstrated that AS 195 film-coated tablets were effective and well tolerated in outpatients with chronic venous insufficiency grade I or II supervised in a setting of private practices. No change of the safety profile has been noted.

[Monsieur & Van Snick 2006]: A small, open observational clinical study was conducted in **39** patients suffering from Chronic Venous Insufficiency (CVI), grade I or II according to the Widmer

Samsung Bioepis Exhibit No. 1002
Page 1124

classification, (grade 2 to 4 of the international CEAP classification). Patients were treated with 180 mg of RVLE (AS 195) twice daily (360 mg in total) for six weeks. The parameters investigated were objective measurements of lower leg volume and the circumference of the leg as well as subjective criteria such as heaviness and pain in the leg. A clear and significant improvement of all parameters was observed after two weeks of treatment. This effect was still present and increased slightly after four and six weeks in all objective and subjective parameters tested in this study.

Assessment This study has shown a fast onset of action and an efficacy of the AS 195 extract in chronic venous insufficiency. Adverse events/side effects: not specified. In general, the treatments were well tolerated. Three patients prematurely stopped the trial for reasons not linked to the treatment or to the disease. No change of the safety profile.

Clinical studies with grapevine leaf extract (4-6:1, extract solvent: water) (AS 195)

Study ID	Study Dates Persons	Design Control type Study objective	Study &, Ctrl Drugs Dose, Route, & Regimen	Subjects per Arm treated / completed	Duration	Gender M/F Median Age (Range)	Diagnosis Inclusion Criteria	Primary Endpoint	Outcome
Kiesewetter, 2000	April Sept-1998 **260**	randomized, double-blind, parallel, dose response Placebo Efficacy, tolerability, safety	AS 195 caps 180mg, *per os* 2-0-0 AS 195 capsules 360 mg, *per os* 2-0-0 Placebo	360 mg : 86/84, 720 mg :64/79 87/80	12 wks	24/62 56.2±12.4 years (mean±SD) 27/57 55.7±13.8 years (mean±SD) 20/67 59.2±11.5 years (mean±SD)	CVI Grade I, II according to Widmer	Baseline adjusted changes of lower limb volume assessed by water displacement plethysmography	Positive
Schaefer & Petrini 2004	2004 **247**	randomized, double-blind, parallel Placebo Efficacy, tolerability	AS 195 tablets 360 mg, *per os* 1-0-0 Placebo 1-0-0	360 mg : 121/117 126/123	12 wks, 2 wks single blind placebo	20/99 53.0 years (20–83 years) 15/111 53.0 years (23–79 years)	CVI CEAP-Scores 3 or 4a	Baseline adjusted changes of lower limb volume assessed by water displacement plethysmography	Negative
Schaefer & Petrini 2003	2003 **179**	randomized, double-blind, controlled cross over trial Placebo Efficacy	AS 195 tablets 360 mg, *per os* 1-0-0 Placebo 1-0-0	360 mg	17 weeks	No data	CVI Grade I, II according to Porter	Baseline adjusted changes of lower limb volume Changes in CVI symptoms	Negative
Vix et al. 2006	2006 **211**	randomized, double-blind, parallel Placebo Efficacy, tolerability	AS 195 tablets 360 mg, *per os* 1-0-0 Placebo 1-0-0	360 mg : 103/98 99/95	12 wks	32/71 61.0 years (29–86 years) 26/73 61.0 years (30–87 years)	CVI CEAP Scores 3 or 4a	Baseline adjusted changes of lower limb volume assessed by water displacement plethysmo-graphy	Negative
Kalus 2004	2002, completed **71**	randomized, double-blind, cross-over Placebo	AS 195 tablets 360 mg, *per os* 1-0-0	360 mg : 70/70	6 wks	16/55 65.2±7.7 years (mean±SD) (32–76)	CVI Grade I, II according to Widmer	resting flux, change from baseline measured by Laser Doppler fluxmetry	Increase of microvascular blood flow

Samsung Bioepis Exhibit No. 1002
Page 1125

Study ID	Study Dates Persons	Design Control type Study objective	Study &, Ctrl Drugs Dose, Route, & Regimen	Subjects per Arm treated / completed	Duration	Gender M/F Median Age (Range)	Diagnosis Inclusion Criteria	Primary Endpoint	Outcome
		Efficacy, tolerability	Placebo 1-0-0						
Schaefer et al. 2003	June 2001 Oct-2001 **65**	Open/ Observational Uncontrolled Tolerability, safety, Efficacy,	AS 195 tablets 180 mg, *per os* 2-0-0	360 mg : 65/59	6 wks	7/58 M: 65.9 years (mean) (56-82 years) F: 56.6 years (mean) (25-80 years)	CVI Grade I, II according to Widmer	Incidence and intensity of adverse events; Changes in CVI symptoms as secondary endpoint	Safety study Positive
Monsieur 2006	2006 **39**	Open Tolerability, safety, Efficacy	AS 195 tablets 180 mg, *per os* 2-0-0	360 mg	6 wks		CVI Grade I, II according to Widmer	Baseline adjusted changes of lower limb volume Changes in CVI symptoms	Positive
Limoni 1996	Dec-1996 May 1997 **105**	randomized, double-blind, parallel, dose response Placebo Multicentre Tolerability, Safety	AS 195 caps. 180 mg, *per os* 1-0-2 Placebo 1-0-2	540 mg : 72/64 33/27	4 wks	6/66 45.2 years (18.4-67.9 years) 4/29 36.7 years (25.6-70.6 years)	CVI Grade I, II. III according to Widmer	Safety Global improvement	Safety

4.2.3. Clinical studies in special populations (e.g. elderly and children)

No information available.

4.3. Overall conclusions on clinical pharmacology and efficacy

The clinical efficacy of an extract from red vine leaves (RVLE; AS 195) was investigated in double blind, placebo-controlled studies [Kiesewetter et al. 2000; Kalus et al. 2004] and in open observational studies [Schaefer et al. 2003; Monsieur & Van Snick, 2006] during treatment of chronic venous insufficiency. In these studies, the RVLE was showed an acceptable clinical efficacy and tolerability, while there were some others with negative results [Schaefer & Petrini 2003; 2004; Vix et al. 2006]. Treatment with RVLE has been shown to lead to a reduction of leg volume, measured using both water plethysmography and calf and ankle circumference, and of the subjective symptoms investigated in the studies reviewed.

The extract AS 195 was consistently more effective than placebo in reducing leg oedema and improving symptoms of Chronic Venous Insufficiency (CVI) across most efficacy parameters evaluated, even though not always a statistically significant superiority could be shown.

Samsung Bioepis Exhibit No. 1002
Page 1126

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

The safety profile of vine leaf extracts can be described as acceptable from all clinical studies in Chronic Venous Insufficiency (CVI) patients and from its use from products on the market. The safety results obtained from the clinical studies conducted so far show that the oral use of vine leaf extracts are well tolerated by most patients. No drug-related serious adverse events were reported during the clinical trials, and when reported that were mild and transient. Seven non-serious adverse events (constipation, dermatitis, headache, hair thinning, menometrorrhagia, urticaria) or moderate (erythematous rash) of mild intensity leading to patient withdrawals, were suspected to be related to the active trial medication.

5.2. Patient exposure

The controlled studies evaluated 1073 patients in total [Kiesewetter et al. 2000; Kalus et al. 2004; Schaefer & Petrini 2004; Vix 2006, Limoni 1996]. The open studies, evaluated by [Schaefer et al. 2003] and by [Monsieur and Van Snick, 2006] comprised 104 patients.

5.3. Adverse events

No health hazards or side effects are known in conjunction with the proper administration of designated therapeutic dosages. A reversible inhibition of intestinal enzyme activity (alkaline phosphatase, sucrose and dipeptidyl peptidase) was demonstrated in animal models [Tebib, 1994; PDR for Herbal Medicines, Thomson, 2004].

Brito et al. [2008]; Mur et al. [2006]: Vine pollen allergies have been reported with *Vitis vinifera* pollen and an extract thereof In Castilla-La Mancha, Spain, the area with the highest density of vineyards in the world. Two cases of allegic reactions to *Vitis vinifera* pollen and grape have been previously reported. The aim of a prospective study by Brito et al. [2008] was to determine the clinical relevance and biochemical characteristics of vine pollen in the Spanish province of Ciudad Real. The authors designed a prospective study of patients treated in the allergy units from Puertollano and Ciudad Real for respiratory symptoms of 4 months' duration in the year 2000. Skin prick tests with a standard aeroallergen battery and *V. vinifera* pollen extract were performed on all patients. The authors also performed conjunctival and bronchial provocation tests and serum specific IgE and sodium dodecyl sulfate-polyacrylamide gel electrophoresis immunoblotting on the patients who tested positive for *Vitis vinifera* pollen.

The results of this prospective study show that *V. vinifera* pollen has a moderate clinical relevance from an allergic point of view, particularly affecting those subjects who are exposed to it during the working hours and those who perform leisure activities close to vine fields. During a period of four months, the authors performed skin prick tests to vine pollen in patients who attended the outpatient clinic with suspected respiratory allergy, finding sensitisation to *Vitis vinifera* pollen in 9 of the 200 patients seen (98 patients had allergy to other pollen). In conclusion, in areas with a high density of vineyards, vine pollen can reach midrange air concentrations and could be the cause of hay fever in those individuals with the highest level of exposure [Brito et al. 2008; Mur et al. 2006].

Assessment: In summary, there is no new safety specific information provided. No change of the safety profile is suggested.

Reports on immunoglobulin E (IgE)-mediated allergic reactions to grapes and wine are limited in the literature. Nevertheless, grapes are widely grown and consumed in Mediterranean countries. The

Samsung Bioepis Exhibit No. 1002
Page 1127

object of a prospective study by Kalogeromitros et al. [2005] was to present clinical features, *in vivo* and *in vitro* allergy testing, and human leukocyte antigen (HLA) serotyping in patients with recurring reactions to grapes and grape products. Eleven unrelated Greek patients, six men and five women (aged 16-44 years; mean, 26.9 years) were enrolled based on a documented history of IgE-mediated reactions to grapes, wine, or other grape products. Their evaluation included full history, reaction severity, clinical examination, skin-prick tests with food allergens and molds, serum IgE, specific IgEs to the same allergen battery, and HLA typing. Patients reported 35 grape-induced episodes of anaphylaxis ranging from moderate to severe. The authors described several cases of repeated IgE-mediated reactions to grapes, vine and other grape products. Similar clinical manifestations in different courses of grape allergen, almost identical prevalent co-sensitisations to other already known allergenic fruits, and the first determination of HLA antigens in grape-allergic patients are the core of this study.
The authors suggest that allergy to grapes may not be as uncommon as generally believed in the literature and should be considered as a possible offending cause in certain episodes of food-induced anaphylaxis probably in genetically at-risk individuals. However, allergic reactions to *Vitis vinifera* are known and reported in rare cases [Kalogeromitros et al. 2005].

Assessment: If hypersensitivity reactions are labelled correctly in the PIL and SPC, there is no change of the safety profile of medicinal products.

[Crowell et al. 2004]: Resveratrol-Associated Renal Toxicity. Resveratrol, (3, 5, 4'-trihydoxystilbene) a compound found in grapes, mulberries, and peanuts, has antimycotic, antiviral, and beneficial cardiovascular and cancer preventive activities. To evaluate the potential toxicity of resveratrol, rats were administered by gavage 0, 300, 1000, and 3000 mg trans-resveratrol per kilogram body weight per day for 4 weeks. Most of the adverse events occurred in the rats administered 3000 mg per kilogram body weight per day. These included increased clinical signs of toxicity; reduced final body weights and food consumption; elevated BUN (blood urea nitrogen), creatinine, alkaline phosphatase, alanine aminotransferase, total bilirubin, and albumin; reduced hemoglobin, hematocrit, and red cell counts; and increased white cell counts. Increases in kidney weights and clinically significant renal lesions, including an increased incidence and severity of nephropathy, were observed. Diffuse epithelial hyperplasia in the bladder was considered, equivocal and of limited biological significance. No histological effects on the liver were observed, despite the clinical chemistry changes and increased liver weights in the females. Effects seen in the group administered 1000 mg resveratrol per kilogram body weight per day included reduced body weight gain (females only) and elevated white blood cell count (males only). Plasma resveratrol concentrations in blood collected 1 h after dose administration during week 4 were dose related but were relatively low given the high dosage levels; conjugates were not measured. Under the conditions of this study, the no observed adverse effect level was 300 mg resveratrol per kilogram body weight per day in rats (corresponding to 21 g in a 70 kg human) [Crowell et al. 2004].

Assessment: These results cannot be transferred to medical products containing Vitis vinifera or extracts thereof.

In summary, there is no safety specific information that is relevant for medical products. The reported adverse events / side effects were mild to moderate. The majority of the above mentioned events are considered to be related to underlying diseases, incidental concomitant disorders or other coincidences but not causally related to *Vitis vinifera*. From these published adverse events, no change of the safety profile of *Vitis vinifera* can be concluded. According to current knowledge, gastrointestinal disorders and hypersensitivity reactions should be labelled in the PIL and SPC.

The Periodic Safety Update Report for *Vitis vinifera* has been compiled within the joint BAH PSUR project. It summarizes safety data from 01/Nov/2003 to 01/Sep/2008 [BAH 2008].

Samsung Bioepis Exhibit No. 1002
Page 1128

In the report period no new safety relevant issues were found in the literature. There were no reports, clinical or experimental studies identified that give reason for a change in the assessment of the safety and benefit/risk ratio of *Vitis vinifera*.

5.4. Serious adverse events and deaths

The case of a 51 years old female patient is reported. She was hospitalized due to an acute icterus, which had developed over 15 days and was associated with asthenia, nausea and anorexia. Beyond the mucocutaneous icterus, the clinical examination did not show any abnormality, especially no chronic hepatopathy. The laboratory parameters suggested a cytolysis due to an ALAT value that was twelve times higher than normal, γ-GT and alcalic phosphatase plasma concentrations two times higher than normal and total bilirubine of 87 µmol/L. Normal findings in the abdominal ultrasonic examination lead to the diagnosis of an acute hepatitis. The patient has a history of euthyroidism. The patient did not consume alcohol and was in a good nutritional condition. The etiologic examination of this hepatitis was negative for infections, immunological and metabolic causes. A hepatic punction-biopsy, revealed a discrete cholangitis with focal ductal hyperplasia, a major cholestasis with numerous biliary thromboses and intrahepatocytic biliary pigments, a focal hepatocellular necrosis, a lymphohistiocytic infiltrate of weak intensity without significant fibrosis. Altogether this suggested an acute cholestatic hepatitis with either viral or toxic origin. The review of the medical questionnaire revealed sporadic intake of 1 g of acetaminophen and two repeated intakes in an interval of one week, when the first symptoms had appeared. Additionally the patient took *Vitis vinifera tinctoria* (extract of red wine leaves) and Fumitory (extract of *Fumaria officinalis*) daily for two months, as "summer cure" for the treatment of heavy legs and as a stimulant. She stopped the intake of these two products three weeks prior to hospitalization, when the first general symptoms appeared. The patient had already taken *Vitis vinifera tinctoria* at the same time one year before. Fumitory was used for the first time. According to the official method used at the pharmacovigilance centres in France, this case is classified as plausible with a chronological C2 and a semiotic S3 score of two products concomitantly used with paracetamol [Bonnet et al. 2007].

Assessor's comment

This case is classified as serious because of a significant medical reaction including hospitalisation. A causal relationship with *Vitis vinifera* cannot be excluded (plausible temporal relationship). However, the used herbal products are insufficiently described; additionally relevant information on the dosage are lacking. Moreover, the concomitant use of acetaminophen must be considered. Acetaminophen is well known for its hepatotoxicity.

5.5. Laboratory findings

None reported.

5.6. Safety in special populations and situations

The product is not suitable for patients with known hypersensitivity against the herbal substance, the plant family, the herbal preparation or to the excipients of the final product.

5.7. Intrinsic (including elderly and children) /extrinsic factors

Grapevine leaf is not intended for use in children, while no restrictions are known for its use in elderly.

Samsung Bioepis Exhibit No. 1002
Page 1129

5.8. Drug interactions

No drug interactions have been reported.

5.9. Use in pregnancy and lactation

Grapevine leaf should not be used during pregnancy and lactation as there are not data available.

5.10. Overdose

No information.

5.11. Drug abuse

No information.

5.12. Withdrawal and rebound

No information.

5.13. Effects on ability to drive or operate machinery or impairment of mental ability

No information.

5.14. Overall conclusions on clinical safety

The safety profile of grapevine leaf extracts can be judged as good from clinical studies and from its long term use and market availability. The available literature, on pharmacological and toxicological studies, does not give rise to safety concerns. A special focus was set on the polyphenolic constituents of the extract (proanthocyanidins, flavonoids) that can be linked with the pharmacological effects. The proof of an acceptable safety is not only given by the period of time over which this extract has been used and by the quantitative aspects of their use but also by PSURs data and the safety data obtained during the clinical trials.
Overall, *Vitis vinifera,* leaves can be considered as safe in herbal medicinal products with a positive benefit/risk ratio.

6. Overall conclusions

Well-established use

The clinical efficacy and safety of the dry aqueous extract of grapevine leaves (4-6:1) has been demonstrated in appropriately designed clinical trials and is supported by extensive use of the product in the market since 1999.

The above referred extract showed an influence to the microcirculation and transcutaneous oxygen pressure at the predominantly affected perimalleolar area of the leg in Chronic venous insufficiency (CVI) patients who were treated for six weeks. Moreover, the assayed grapevine leaves extract has shown to lead to a reduction of the volume of oedema and to improvement of the typical subjective symptoms of CVI such as tired, heavy and swollen legs or pain and tension in the legs.

Three double-blind, placebo controlled trials investigated the change in leg volume as measured by water plethysmography as the primary endpoint, another also the changes in ankle and calf

Samsung Bioepis Exhibit No. 1002
Page 1130

circumference in the secondary endpoints investigated. In several of the clinical trials objective and subjective symptoms of CVI appeared to be effectively reduced, while there were some others giving negative results such as the ones of Schaefer & Petrini 2004 and Vix et al. 2006. The trials were performed according to ICH-GCP.

An additional placebo-controlled and open safety and tolerability trial provided supportive evidence for the efficacy.

This extract can therefore be regarded as an active substance with a well-established medicinal use.

The use of herbal medicinal products prepared with this extract is not recommended during pregnancy and lactation and should not be taken in children and adolescents under 18 years of age, due to the lack of adequate data.

The proposed indication is:

- Herbal medicinal product for treatment of chronic venous insufficiency, which is characterised by swollen legs, varicose veins, a feeling of heaviness, pain, tiredness, itching, tension and cramps in the calves.

Traditional use

Other extracts and herbal preparations from grapevine leaves have been widely used. The safe use of the extract can be stated on the basis of the well-known, long-lasting and traditional use of preparations of grapevine leaves in the folk medicine and as registered medicaments.. Based on the evaluation and assessment of the present documentation of the clinical efficacy and safety, the herbal substance for use as herbal tea or in other oral dosage forms, and the aqueous extracts of grapevine leaf are acceptable as traditional herbal medicinal products.

Sufficient data are available to develop a Community monograph on the well established and traditional use of *Vitis vinifera* L., leaves. The indications are suitable for self-medication. The proposed indications are:

- Traditional herbal medicinal product to relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances.
- Traditional herbal medicinal product for symptomatic relief of itching and burning associated with haemorrhoids.
- Traditional herbal medicinal product for symptomatic treatment of cutaneous capillary fragility.

The use of the traditional herbal medicinal products is not recommended during pregnancy and lactation and should not be taken in children and adolescents under 18 years of age.

The minimum required data on mutagenicity (Ames test) are available for herbal preparations of grapevine leaves. **The inclusion in the Community list of traditional herbal substances and preparations is recommended.**

Annex

Samsung Bioepis Exhibit No. 1002
Page 1131

VOSSIUS & PARTNER
Patentanwälte Rechtsanwälte

VOSSIUS & PARTNER * POB 86 07 67 * 81634 München * Germany

EPO - Munich
58

11. Nov. 2014

European Patent Office

80298 MUNICH

T1897/14-3.3.04
(05 71 2607.0 / 1720571)
Patentee: Alexion Pharmaceuticals, Inc.
Opposition by: Pfizer Inc.
Our Ref.: M2367 OPP(EP)

München, November 11, 2014
WA/FLG

Further to the appeal filed on September 8, 2014, against the decision of the Opposition Division to revoke the above-referenced European patent entitled "Method for treating haemolytic disease" issued on July 1, 2014, we now provide the grounds of appeal in accordance with Article 108 EPC.

It is requested that the patent is maintained for all designated contracting states based on the enclosed Main Request. Its subject-matter is patentable within the terms of the EPC. If the Board of Appeal does not see itself in a position to comply with our main request on the sole basis of our written submissions, we request consideration of auxiliary request 1.

The main request and auxiliary request 1 are identical to the main request and auxiliary request 1 dealt with in the impugned decision.

Should the Board be minded not to accept patentability of any of the enclosed requests, we request that the Board exerts its discretion to allow further claim amendments.

PATENTANWÄLTE - DEUTSCHLAND
EUROPEAN PATENT ATTORNEYS
EUROPEAN TRADEMARK ATTORNEYS
DR. DIETER HEUNEMANN, Dipl.-Phys.
DR. GERHARD HERMANN, Dipl.-Phys.
JOSEF SCHMIDT, Dipl.-Ing.
DR. HANS-RAINER JAENICHEN, Dipl.-Biol.
DR. ALEXA V. UEXKÜLL, M.Sc.
DR. RUDOLF WEINBERGER, Dipl.-Chem.
DR. JOACHIM WACHENFELD, Biol.
DR. FRIEDERIKE STOLZENBURG, Dipl.-Biol.
RAINER VIKTOR, Dipl.-Ing.
DR. NATALIA BERRYMAN, Dipl.-Chem.
DR. JÜRGEN MEIER, Dipl.-Biol.
DR. STEFAN FICKERT, Dipl.-Chem., LL.M.
ARNOLD ASMUSSEN, Dipl.-Ing.(FH)
ELARD SCHENCK ZU SCHWEINSBERG, Dipl.-Ing., LL.M.
DR. DIRK HARMSEN, Dipl.-Chem.
DR. KATHARINA HAAS, Dipl.-Chem.
BARBARA BRÜSZAT, Dipl.-Phys.
DR. SUSANNE FISCHER-KRUMMER, Apothekerin
DR. JÖRG HAHN, Dipl.-Chem.
DR. CHRISTOPH EISERMANN, Dipl.-Phys.
DR. MICHAELA WESSE, Apothekerin
DR. OLIVER SAHR, Dipl.-Phys.
DR. AXEL LEINE, Dipl.-Phys.
DR. CHRISTIAN SCHLORB, Dipl.-Biochem.
OSWIN RIDDERBUSCH, Dipl.-Biochem.
DR. MAURIZIO DI STASIO, Dipl.-Biol.
CLAUS PETER PIETRUK, Dipl.-Phys. *
RAPHAEL SALZER, Dipl.-Ing.

DR. PAUL TAUCHNER, Dipl.-Chem. (Of Counsel)
DR. PETER A. RAUH, Dipl.-Chem. (Of Counsel)

PATENTANWALT - SCHWEIZ
EUROPEAN PATENT ATTORNEY
DR. WERNER BASTIAN, Dipl.-Biol. *

EUROPEAN PATENT ATTORNEYS
DR. URSULA HOFMANN, Dipl.-Chem.
DR. PETER EINMAYR, Dipl.-Chem.
DR. OLAF MALEK, Dipl.-Biol.
DR. ROGER ABSEHER, Biochem.
DR. DANIEL EISENBARTH, Dipl.-Biol.
DANA KRÄMER, Ph.D., Dipl.-Biochem.
KIMIO TAKAHASHI 1)
DR. ULRICH SENTNER, Dipl.-Biol.
DR. ALEXANDER SCHWENDEMANN, Dipl.-Biol.
NAMI SAEKI 1)
DR. STEFAN MÜLLER, Dipl.-Biol.
DR. FLORIAN GRASSER, Dipl.-Biol.
RICHARD ENMON, Ph.D., J.D. 5)

PATENTANWALT - DEUTSCHLAND
DR. ANDREAS M. ROBINSON, Dipl.-Biol.

RECHTSANWÄLTE
DR. JOHANN PITZ
DR. THURE SCHUBERT
DR. MATHIAS KLEESPIES, LL.M.
SIMONE SCHÄFER
PAUL KRETSCHMAR, LL.M. 2)
DR. MARCUS V. WELSER, LL.M. 2)
CHRISTIAN HUFNAGEL, LL.M. 3)
JENNIFER CLAYTON-CHEN
DR. GEORG ANDREAS RAUH
NILS BINGS, LL.M.
CHRISTIAN-LEOPOLD ZAPP

ACHIM BENDER (Of Counsel)
WOLFGANG VON MEIBOM (Of Counsel)

CONSULTANTS
DR. CHRISTIAN GUGERELL 4)
YOSHIKAZU ISHINO
JAEHYUK CHOI, LL.M. 6)
DONGLAI XU

1) Japanese Patent Attorney
2) Fachanwalt für gewerblichen Rechtsschutz
3) U.S. Attorney-at-Law (New York)
4) European Patent Attorney
5) Registered U.S. Patent Attorney
6) Korean Patent Attorney

MAIN OFFICE
SIEBERTSTR. 3
81675 MÜNCHEN/GERMANY

POSTAL ADDRESS:
POB 86 07 67
81634 MÜNCHEN/GERMANY

Tel.: +49-(0)89-413 04-0
Fax: +49-(0)89-413 04-111
Fax trademarks: /-400

BERLIN OFFICE
JOACHIMSTHALER STR. 34
10719 BERLIN/GERMANY

Tel.: +49-(0)30-340 609-501
Fax: +49-(0)30-340 609-512

Partnerschaftsregister Amtsgericht München PR 89

BASEL OFFICE (CH) *
NADELBERG 3
4051 BASEL/SWITZERLAND

Tel.: +41-(0)61 560 1490
Fax: +41-(0)61 560 1488

HR Firmennummer CH-270.9.001.271-9

E-Mail:
patents@vossiusandpartner.com
trademarks@vossiusandpartner.com
litigation@vossiusandpartner.com

www.vossiusandpartner.com

Liste aller Partner i.S.d. PartGG:
www.vossiusandpartner.com/partner

If the Board does not see itself in a position to comply with any of our claim requests on the sole basis of our written submissions, we request oral proceedings in accordance with Article 116(1) EPC.

An adapted description can be provided at short notice.

I. MAIN REQUEST

1. INVENTIVE STEP (Article 56 EPC)

1.1. The Opposition Division (OD) concluded in section 2.27 of the contested decision that the skilled person is taught by D5 to reduce the dosage interval to solve the problem of complement breakthrough in paroxysmal nocturnal hemoglobinuria (PNH) patients receiving the priming dosage described in D6. The OD took the position that the skilled person would have reduced the interval "slightly", to 12 days to resolve the symptoms, as allegedly suggested by D5.

The OD stated by referring to T1979/09 that the replacement of one dosing regimen with another is a matter of "routine experimentation", regardless of the nature of the agent(s) being administered, the condition being treated, the relevant patient population or the complexity of the dosing regiment being "replaced".

1.2. Contribution of the invention to the art

Claim 1 of the main request is directed to eculizumab for use in treating a patient afflicted with paroxysmal nocturnal hemoglobinuria (PNH), wherein eculizumab is to be administered by intravenous infusion to the patient under the following dosing schedule:

(i) 600 mg each week for four weeks;
(ii) 900 mg 1 week later;
(iii) 900 mg on a biweekly basis; and thereafter
(iv) 900 mg every 12 days,

wherein said patient exhibits breakthrough in serum hemolytic activity when treated under a dosing schedule according to items (i) to (iii).

Samsung Bioepis Exhibit No. 1002
Page 1133

Hence, the claims of the main request cover a specific and narrow four-stage treatment schedule for PNH patients with the anti-C5 antibody eculizumab.

As is well-known PNH is a rare, genetically acquired, life-threatening disease of the blood characterized by destruction of red blood cells by the complement system. The primary clinical manifestations of PNH range from hemolytic anemia, thrombosis or other deficiencies in hematopoiesis PNH patients may develop thrombosis (a blood clot) at some point in their illness, which is the main cause of severe complications and death in PNH. In fact, **about 50% of the patients die within 10 years** after diagnosis of the disease.

In 2007, eculizumab was approved for the treatment of PNH and dramatically changed the outlook for patients with PNH. Depending on the underlying extent of bone marrow failure certain patients can experience a breakthrough in hemolytic activity toward the end of the dosing interval (see discussion in paragraphs 76-78 of the patent). In order to ensure that PNH patients benefit from the treatment with eculizumab to the desired effect that **complement breakthrough is prevented** it is of utmost importance that eculizumab is administered in an appropriate dosage regimen.

The four-stage treatment schedule set forth in claim 1 of the main request is tailored to this appropriate dosage regimen of eculizumab. Eculizumab has the trade name Soliris®. The leaflet of Soliris® has been submitted as D15 during the opposition hearing. The claimed four-stage treatment schedule can be taken from sections 2.1 and 5.6 of D15.

The claims are therefore directed to the exact dosage regimen of eculizumab which saved lives of PNH patients and will do so in the future. These PNH patients have a decreased or even no need for blood transfusions, as well as a significantly decreased incidence of thrombosis. The contribution of the present invention to the art therefore should not be underestimated.

1.3. Difference between the closest prior art and the main claim

The claims of the main request provide for an improved treatment schedule of PNH patients showing complement breakthrough when previously treated using

Samsung Bioepis Exhibit No. 1002
Page 1134

the established dosage schedule (i.e. steps (i) to (iii) of claim 1). As D5 addresses the problem of complement breakthrough, D5 has been considered as closest prior art.

D5 is a conference abstract authored by inventors of the revoked patent. The following is an excerpt of D5:

> "We previously reported that eculizumab was very effective at acutely controlling intravascular hemolysis in a 3-month Pilot study of transfusion-dependent PNH patients. We now report that all 11 patients from the initial study entered an extension trial of 900 mg eculizumab by i.v. infusion over 30 minutes every other week for one year. [...] Two patients experienced transient breakthroughs in complement activity associated with LDH, hemoglobinuria and mild symptoms; these resolve immediately upon next dose of eculizumab and further breakthroughs were prevented by a slight decrease in dosing interval." (emphasis added)

Details on the 3-months pilot study as well as the slightest information on what is meant by a "slight decrease" are absent from D5. The difference between D5 and the claims is therefore two-fold:

(A) D5 is silent about the dosage schedule applied prior to the biweekly administration of 900 mg (i.e. steps (i) and (ii) of claim 1).

(B) D5 is silent about the feature of administering 900 mg every 12 days (step (iv) of claim 1 of the main request).

In view of D5 the technical problem underlying the claims may be seen as the provision of a complete eculizumab dosage regimen which is capable of preventing complement breakthroughs in all PNH patients, including those PNH patients which suffered from a complement breakthrough in the extension trial; see also section 2.15 of the impugned decision.

1.4. Distinguishing feature (A)

1.4.1. Two possible dosage schedules – both being applied prior to the biweekly administration of 900 mg (i.e. step (iii) of claim 1) - can be found in the prior art; see sections 2.18 and 2.19 of the impugned decision. While D6 describes a schedule which comprises steps (i) and (ii) of claim 1, D3 and D4 only describe

Samsung Bioepis Exhibit No. 1002
Page 1135

step (i) but not step (ii). The skilled person is consequently confronted with the necessity to choose from two options, being a two step or a one step dosing schedule prior to step (iii) of claim 1.

In its assessment of inventive step the OD found that the skilled person would have reverted to D6 as secondary reference, since only D6 but neither D3 nor D4 discloses a dosing schedule comprising the administration of (i) 600 mg eculizumab each week for four weeks, (ii) 900 mg eculizumab 1 week later and (iii) 900 mg eculizumab on a biweekly basis as set forth in claim 1. The OD justified its position that the skilled person would have preferably turned to D6, by stating that D6 allegedly describes "the most recent and detailed teaching"; see section 2.25 of the impugned decision.

1.4.2. The skilled person can be defined in the present case as a medical doctor, more specifically a clinician or a team of clinicians specializing in the treatment of haemological disorders.

The oral communication corresponding to the abstract of D3 has been presented in April 2003, the abstract D4 is from November 2002, and the abstract D6 dates September 2003. Thus, from the outset, all three references were published within the same one year period. D6 would not necessarily have been considered more recent in any meaningful way which would have led the skilled artisan to have favored this reference. Moreover, while D6 mentions step (ii) of claim 1, neither D3 nor D4 mentions this step. Hence, the teachings of D3 and D4 are consistent, while D6 teaches something else.

Importantly, D6 assigns no particular relevance to the presence or absence of step (ii). However, step (ii) 900 mg eculizumab 1 week later – is **essential** in order to provide an effective and optimal dosage regimen, as evidenced by section 2.1 of D15 (i.e. the post-filing product leaflet of Soliris®). Indeed, this could only be known through hindsight. In this respect reference is made to section 4 of the declaration D14. The technical expert Dr. Bell explains in section 4 that

> "This dose is important because, without it, the loading dose specified by step (i) does not provide an adequate baseline of complement inhibition that can be maintained by the maintenance dose of step (iii) or (iv)"

Samsung Bioepis Exhibit No. 1002
Page 1136

It follows that the omittance of step (ii) puts the PNH patients at an undesired increased risk of developing complement breakthrough.

In view of the contents of D3, D4 and D6 is not entirely clear whether D3 and D4 on the one hand and D6 on the other hand describe the same clinical study on PNH patients with eculizumab or two different studies. At face value and in view of the discussed differences there is some evidence that there are two different studies. However, even if considering that all three documents describe the **same** study all PNH patients would already have received the prior dosing schedule prior to the submission of the three abstracts by their authors. The OD's assumption that D6 describes something more recent and more detailed than D3 or D4 is therefore not correct under the assumption that the same study is described.

Irrespectively of whether assuming that the same clinical study or two different studies are described, the conclusion of the OD that the skilled person immediately and unquestionably knows that only D6 but not D3 or D4 describe the correct prior dosing schedule can only be drawn based on hindsight.

The OD in selecting D6 as a secondary reference obviously applied an "ex-post facto" analysis. As the Board is well aware, the Boards of Appeal have ruled that an "ex-post facto" approach when assessing inventive step is impermissible (see, for example, T 24/81, T 564/89, T 645/92 or T 795/93).

A correct application of the problem and solution approach has to avoid the inadmissible "ex-post facto analysis", the OD's biased selection of D6 as secondary reference already indicates that the decision to revoke the patent for an alleged lack of inventive step is not correct.

1.5. Distinguishing feature (B)

As it can be taken from the examples of the patent at paragraph [0073]

> "During the extension study, 2 patients did not sustain levels of eculizumab necessary to consistently block complement. [...] A reduction in the dosing interval from 14 to 12 days was sufficient to maintain levels of eculizumab

Samsung Bioepis Exhibit No. 1002
Page 1137

> above 35 µg/ml and effectively and consistently blocked serum hemolytic activity for the remainder of the extension study for both patients."

It will be detailed below that the OD's assumption that the skilled person in view of D5 – even if considering D6 as the "preferred" secondary reference - would have arrived at administering 900 mg every 12 days (step (iv) of claim 1 of the main request) without inventive skills is based on unjust oversimplifications.

1.5.1 *Optimizing the eculizumab dosing schedule is not a matter of routine*

First, there is nothing routine about treating PNH patients with eculizumab, which is a humanized monoclonal antibody that binds to complement protein C5 and prevents its cleavage into C5a and C5b.

As is well-known, the complement protein C5 is a plasma protein. In order to fulfill its function it must be present throughout the body and at a relatively high concentration of 75 µg/ml (0.4 µM) in normal serum; see paragraph [0031] of the patent.

The skilled person is particularly cautious when it comes to interfering with the complement system which is a key component of the human immune system. This is also expressed in D13:

> "Since complement activation has both positive and negative effects, the risk associated with complement inhibition is probably not negligible and long term studies will be necessary to assess possible risks. In particular, a theoretical concern is the role that C5 plays in hepatic regeneration and in combating granulomatous infections such as tuberculosis. Long term studies will determine whether C5 inhibition could have deleterious effects in these processes." (see page 1021; left hand column; top)

The skilled person would have correctly proceeded very cautiously when either increasing the dose or reducing the dose of eculizumab. For example, D15 carries a prominent "Black Box" warning at the top of the first page. The warning states the following:

> "Life-threatening and fatal meningococcal infections have occurred in patients treated with soliris and may become rapidly life-threatening or fatal if not recognized and treated early"

Samsung Bioepis Exhibit No. 1002
Page 1138

Furthermore, at the time the invention was made, those skilled in the art were fearful that treatment with eculizumab, which causes the PNH red cell clone size to increase from 36.7% to 58.9% could leave the patient vulnerable to a massive haemolytic attack if treatment was withdrawn (see paragraph [0069] of the patent). This fear is expressed in D7 as follows:

> "The reduction or cessation of transfusions and control of hemolysis in patients on eculizumab leads to an increase in the proportion of PNH red cells toward the level of PNH neutrophils [...] This raises the concern that stopping eculizumab for any reason might render the patient susceptible to a massive haemolytic attack and profound anemia." (see D7, page 53, lower left column).

Moreover, we refer to the sentence bridging pages 52 and 53 of D7, where it is stated:

> "The only apparent sequela [of treatment with eculizumab] is an increased risk of infection by encapsulated organisms, namely *Hemophilus influenza*, *Neisseria meningitides* and *N. gonorrhoea*." (information in brackets added)

As such, the skilled person would be highly concerned about the possibility of severe bacterial infection, and thus had manifest reasons to be exceptionally cautious in view of the specific facts of the present case, namely the potentially dangerous side effects of the anti-C5 antibody eculizumab.

As the Board is well aware, there is settled case law about the attitude of the skilled person in the field of life sciences. The skilled person is cautious and maintains a conservative attitude. She or he does not take unpredictable risks. This point applies to an exceptional extent and should be emphazised in the present case. Choices which carry minimal risk and thus might be considered worth trying or obvious to try (as they are less dangerous) in a particular context, cannot be equated with choices which carry potentially devastating, life threatening risks. The risks posed in the present case, involving a complex biologic drug, i.e. eculizumab, involved in a fundamental, life-depending pathway would have required a significantly heightened evaluation of the potentially available options.

Samsung Bioepis Exhibit No. 1002
Page 1139

This point is highlighted by a different drug of the same class, alemtuzumab, a humanized anti-CD52 antibody which was first approved in 2001 for refractory B-cell chronic lymphocytic leukemia (B-CLL). Alemtuzumab has exceptional lympholytic activity, driven by complement activation antibody-dependent cytotoxicity, and apoptosis. Accordingly, it was well known that this drug often causes severe side effects, particularly when administered intravenously. For example, as reported at page 6 of the enclosed monograph (BC Cancer Agency Cancer Drug Manual, D16[1]) for alemtuzumab, intravenous administration can cause "serious and potentially fatal complications including myocardial infarction, cardiomyopathy, cardiac arrest, arrhythmias, bronchospasm, and acute respiratory distress". Thus, several pre-treatment protocols involving immunosuppressants are recommended prior to administering alemtuzumab, as well as starting with low doses of the drug (3 mg) and escalating the dosage while carefully monitoring patients. In fact, the subcutaneous route has been studied in an attempt to reduce side effects and make the treatment more manageable (see Hale et al. (2004) *Blood* 224(4):948-955 being enclosed herewith as D17[2]). Thus, like eculizumab, optimizing the dosage for alemtuzumab was anything but routine and is still being explored, even at dosages on a magnitude of 200 to 300 times lower than the presently claimed dosages for eculizumab.

D17 goes on to discuss how alemtuzumab may cause substantial "first dose" reactions as a result of cytokine release when it is given intravenously at 3,18 and 19 days. The standard treatment regimen involves dose escalation and frequent, relatively small doses. A full treatment course for resistant CLL consists of approximately one gram of the antibody (see page 954 of D17). This is in stark contrast to the presently claimed, far higher 600 mg and 900 mg dosages (0.9 g every two weeks) for eculizumab, which would have been considered to hold a much greater risk. Moreover, many factors can affect the immunogenicity of alemtuzumab, including the dose and length of treatment, the underlying disease, prior exposure to chemotherapy, concomitant immunosuppressive drugs, and route of administration or the formulation of the drug (see discussion on page 954 of D17). Again, this highlights the fact that optimizing the dosages of

[1] This document is only submitted for providing the Board with an example of a drug which caused fatal complications due to overdosing.
[2] This document is only submitted for providing the Board with an example of a drug which caused fatal complications due to overdosing.

Samsung Bioepis Exhibit No. 1002
Page 1140

complex, antibody-based therapeutics depends on a number of complex, varying factors, and was far from "routine" at the priority date of the present application, even at dosages far lower than those presently claimed.

Indeed, the authors conclude that alemtuzumab provides a useful therapeutic option for patients with CLL. It has excellent activity as first-line treatment and can give good responses in patients who are unresponsive to conventional chemotherapy. However, "the optimal dose route and regimen are not yet known" (see page 955 of D17).

When C5 regulation is perturbed as in PNH the results can be catastrophic. As discussed in paragraph 5 of the declaration D14 of the technical expert Dr. Bell and paragraphs [0002]-[0005] of the patent, failure to **completely** suppress C5 in PNH leads to hemolysis and thrombosis. As mentioned above, thrombosis is the leading cause of death in PNH patients and 50% of PNH patients die within 10 years.

Solving the problem of completely suppressing C5 requires the use of very large doses of eculizumab. However, modifying large doses of any biologic, especially to increase levels of the drug in a patient's serum (e.g., by increasing dosages or shortening dosing intervals), was a risky and dangerous proposition at the priority date, i.e. 2004, not to mention the added complexity in the case of eculizumab where complement C5 plays a **fundamental role in the human immune system**. At that time, and largely still today, the 900 mg maintenance dose was unique among biologic drugs. The dose for most biologic drugs is less than 300 mg. A typical example is the therapeutic anti-TNF antibody adalimumab which is administered at 40 mg every two weeks (see, for example, http://www.drugs.com/dosage/humira.html).

1.5.2. *Possible paths for optimization*

The contents of the prior art do not create a one-way street situation contrary to the position obviously taken by the OD. As mentioned, adjusting the dosing of a biological which interferes with the complement system was and is a most sensitive issue and cannot be equated with a routine measure. This is simply because there was no routine to be relied upon. D5 does not provide a complete

Samsung Bioepis Exhibit No. 1002
Page 1141

teaching how to prevent complement breakthrough in PNH patients because what a "slight decrease in dosing interval" means is entirely open. Hence, at the priority date different paths for optimization were available, such as a 13.5 day interval or 13 day interval. There was no pointer to a 12 days interval. Rather the skilled person would have shied away from such a significant decrease in the time interval.

In the case of the present invention, it was PK/PD modeling and data that led the inventors to identify the 12 days interval as being the **precise and critical** solution to the problem, among other available, potentially useful options. Paragraph [0078], Figures 4 and 8 of the patent exemplify the pharmacodynamic data driven analysis used to choose 12 days. It is again only through hindsight – i.e. starting from the work of the inventors and physicians conducting the eculizumab clinical trials - to assume that the skilled person could have known that the specific changes to the clinical protocol that resulted in the present claims were safe and highly effective at preventing breakthrough. Importantly, it was not obvious or possible to know in advance whether a dangerous inflammatory event would occur at the increased dose resulting from the shortened dose interval. Nor was it obvious or possible to know in advance whether these new conditions would trigger an increased human anti-drug antibody response. Only by way of inventive work resulting in the presently claimed invention (i.e., a highly specific and unpredictible change in dosing frequency among other options) was this discovered.

The fact that the inventors first performed labor intense and inventive PK/PD modeling before actually applying dosing step (iv) to PNH patients underscores how critical any dosing change of eculizumab is. It is in particular no option to use a "try-and-see" approach in PNH patients, given that such an approach would involve irresponsible risks for the PNH patients.

1.5.3. *D5 fails to render decreasing the dosing interval from 14d to 12d obvious*

Appellant strongly urges the Board to reconsider the patentability of the main request on the grounds of an alleged lack of inventive step. The OD has significantly erred in hinging its decision on the vague statement in D5 of "slightly decreasing" the dosing interval to support the assertion that the claimed invention

Samsung Bioepis Exhibit No. 1002
Page 1142

lacks inventive step. Indeed, the OD offers no basis whatsoever for its conclusion that the skilled person would have automatically equated a "slight decrease" in dosing interval with a decrease of considerably more than 10% (i.e., from 14 days to 12 days). Again, this is an ex-post facto approach.

A change from a 14 day interval to a 12 day interval for a drug administered at 900 mg for maintenance dosing results in a **significant increase** in exposure to the patients of 3.9 grams or **16.7%** of therapeutic antibody annually. The magnitude of this change is not commensurate with the phrase "slight decrease" as suggested in D5 (see section 2.20 of the contested decision) but would have carried great risks.

The OD decision should be reversed in this appeal, because the selection of the 12 day dosing interval for patients experiencing breakthrough required inventive skills. As discussed in the above-cited paragraph [0073] of the patent, the dose decision was made to maintain a minimum target level of 35 µg/ml of eculizumab in the affected patient's serum. This change was supported scientifically by examining the PK/PD data generated in patients with PNH and being treated with eculizumab. The OD's reliance on the statement in D5 trivializes the significance of this non-obvious and precise solution to a clinically devastating problem. As discussed above, the change of dosing interval from 14 days to 12 days to prevent breakthrough was not made by simple process of elimination or a try-and-see approach, but rather by inventive reasoning and a carefully selected scientific approach.

1.5.4. *Decision T1979/09 is not applicable*

In addition, we wish to submit that analogizing the decision T1979/09 to the facts and circumstances of the present patent is incorrect. The claims at issue in T1979/09 were directed to a dietary supplement for preventing or treating discomfort associated with venous insufficiency by administering flavonoids by water extracting crushed and dried red vine leaves. This case is distinguishable from the present matter and the decision states that

> "the skilled person would carry out these steps according to the known standard technologies for the processing of medicinal plants" (see page 17 of T1979/09).

Samsung Bioepis Exhibit No. 1002
Page 1143

It cannot be the case that dose considerations made in the context of suppressing complement protein C5 by administering a high dose of a biologic drug to patients with a potentially fatal disease by intravenous administration are routine experimentation on the level of administering flavonoids extracted from leaves of medicinal plants. The consequences of making a mistake in either of the two situations are certainly in no way equivalent.

Cleavage of complement C5 plays a central role in the immune system; see Rother et al. *Nature Biotechnology* 25 (11) 1256-1264 (2007) which is provided herewith as document D18[3]. There are three distinct complement pathways known as alternative pathway, classical pathway, and lectin pathway and they differ in the mechanism of activation. But no matter the manner of activation, all three pathways converge at the point of C5 cleavage. C5 is cleaved into C5a and C5b. C5a is a potent anaphylatoxin that mediates leukocyte chemotaxis, increases vascular permeability and induces other secondary inflammatory mediators. The simultaneous release of C5b triggers the recruitment of terminal complement components C6, C7, C8 and C9 to form the terminal complement complex which results in lysis and destruction of a target cell. Individuals who are deficient in other complement components such as C1, C2 or C4 develop severe autoimmune symptoms such as Lupus-like syndrome and are at recurrent risk of developing sepsis. Therefore, the complete inhibition of complement C5 was rightly viewed in 2004 as a very uncertain and medically consequential activity.

According to the European Medicines Agency (EMA) in their Assessment Report on *Vitis vinifera* L.

> "Since ancient times, beneficial effects on health have been ascribed to wine and vine leaves, as confirmed by numerous "recipes" reported in Egyptian papyri, the Sumerian Tablets, the writings of Hippocrates of Cos (5th-4th Century BC), Celcus (1st century AD), Galen (130-201 AD) and Paracelsus (1493-1541). (see EMA/HMPC/16633/2009 section 2, Historical Data on Medicinal Use provided herewith as document D19[4]).

[3] This document is only submitted for providing the Board with a short and concise overview of the scientific background of the invention.
[4] This document is only submitted for providing the Board with background information on the case at stake in T1979/09.

Samsung Bioepis Exhibit No. 1002
Page 1144

The incorporation of the flavonoids into in a dose range of 300-800 mg and incorporated into a dietary supplement tablet does not change the fact that the flavonoids were known to be generally safe and beneficial. The flavonoids were orally administered and allowed to diffuse into the blood stream in the digestive process. The EMA found that for red vine leaf converted from a tea administration to a tablet

> "there is no safety specific information that is relevant for medical products." (see EMA/HMPC/16633/2009 section 5.3, Adverse Events).

It follows that the administration of flavonoids has no severe side effect and overdosing flavonoids in a patient is not life-threatening for the patient. By contrast, overdosing the first in class biologic protein drug eculizumab may result in the blocking the complement system of the immune system to an extent that puts the patient in a life-threatening situation. In the first case optimizing of the dosing schedule is a matter of routine, whereas in the latter case it is not.

Thus, we contend that dosing through intravenous administration of a first in class biologic protein drug, at an usually high dose, with the intention of suppressing the activity of an immune system component does involve inventive activity. Where the consequences of dosing changes may be catastrophic, the inventors must invest greater judgment and thought about dose changes or dose interval changes. The inventors for a drug such as eculizumab are evaluating the effects of dose changes on the immune system in a very quantitative fashion. Any statements made in the context of dosing decisions for dietary supplements should not apply with equal force to the clinical trials of a first in class drug like eculizumab. We therefore consider that T1979/09 is not applicable. The facts of the case underlying this decision are different from that of the present case.

1.6. In summary, we submit that the reasons of the OD for revoking the patent for an alleged lack of inventive step are without merit. The Board of Appeal is therefore requested to set the decision of the OD aside and to give the order to maintain the initially granted patent based on the main request.

Samsung Bioepis Exhibit No. 1002
Page 1145

II. AUXILIARY REQUEST 1

In case the Board of Appeal cannot accept that the main request meets the requirements of Article 56 EPC, consideration of auxiliary request 1 is requested. The arguments and facts presented herein above in connection with the main request are equally applicable to the auxiliary request 1.

As compared to the main request claim 1 of auxiliary request 1 further comprises the feature of "reducing cell lysis to below 20% of normal serum complement activity" which is a feature indicating the effective inhibition of haemolytic activity; see page 38, lines 11-13 and Figure 8. The effective inhibition of haemolytic activity is a key advantage being associated with the subject-matter claimed in the main and auxiliary requests. This advantage is expressly incorporated in the main claim of auxiliary request 1.

Dr. Joachim Wachenfeld
European Patent Attorney

Enclosure:
D16 (Monograph from the BC Cancer Agency Cancer Drug Manual)
D17 (Hale et al. (2004) *Blood* 224(4):948-955)
D18 (Rother et al. *Nature Biotechnology* 25 (11) 1256-1264 (2007))
D19 (EMA/HMPC/16633/2009 section 2, Historical Data on Medicinal Use)
Main Request
Auxiliary Request 1

Samsung Bioepis Exhibit No. 1002
Page 1146

T1897/14-3.3.04
(05 71 2607.0 / 1720571)
Patentee: Alexion Pharmaceuticals, Inc.
Opposition by: Pfizer Inc.
Our Ref.: M2367 OPP(EP)

From www.bloodjournal.org by guest on November 10, 2014. For personal use only.

blood

2004 104: 948-955
doi:10.1182/blood-2004-02-0593 originally published online
April 15, 2004

Blood concentrations of alemtuzumab and antiglobulin responses in patients with chronic lymphocytic leukemia following intravenous or subcutaneous routes of administration

Geoff Hale, Peppy Rebello, Lee R. Brettman, Chris Fegan, Ben Kennedy, Eva Kimby, Mike Leach, Jeanette Lundin, Håkan Mellstedt, Paul Moreton, Andy C. Rawstron, Herman Waldmann, Anders Osterborg and Peter Hillmen

Updated information and services can be found at:
http://www.bloodjournal.org/content/104/4/948.full.html

Articles on similar topics can be found in the following Blood collections
Clinical Trials and Observations (3959 articles)
Immunobiology (5230 articles)
Immunotherapy (577 articles)
Neoplasia (4212 articles)

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.
Copyright 2011 by The American Society of Hematology; all rights reserved.

From www.bloodjournal.org by guest on November 10, 2014. For personal use only.

Blood concentrations of alemtuzumab and antiglobulin responses in patients with chronic lymphocytic leukemia following intravenous or subcutaneous routes of administration

Geoff Hale, Peppy Rebello, Lee R. Brettman, Chris Fegan, Ben Kennedy, Eva Kimby, Mike Leach, Jeanette Lundin, Håkan Mellstedt, Paul Moreton, Andy C. Rawstron, Herman Waldmann, Anders Osterborg, and Peter Hillmen

Alemtuzumab is a humanized anti-CD52 antibody licensed for refractory B-cell chronic lymphocytic leukemia (B-CLL), when given intravenously at 30 mg thrice weekly. However, the intravenous route is associated with infusion-related reactions and is inconvenient. We measured blood concentrations in 30 relapsed patients treated with intravenous alemtuzumab and in 20 patients from a previously untreated group who received similar doses subcutaneously. Highest trough samples in the intravenous group were less than 0.5 μg/mL to 18.3 μg/mL (mean 5.4 μg/mL). The cumulative dose required to reach 1.0 μg/mL was 13 mg to 316 mg (mean 90 mg). Higher blood concentrations correlated with the achievement of better clinical responses and minimal residual disease. The highest measured concentrations in the subcutaneous group were similar (0.6 μg/mL to 24.8 μg/mL, mean 5.4 μg/mL). However, the cumulative dose to reach 1.0 μg/mL was higher: 146 mg to 1106 mg (mean 551 mg). No antiglobulin responses were detected in 30 patients given intravenous alemtuzumab whereas 2 of 32 patients given subcutaneous alemtuzumab made substantial anti-idiotype responses. Thus, subcutaneous alemtuzumab achieved concentrations similar to those for intravenous alemtuzumab, although with slightly higher cumulative doses. Subcutaneous alemtuzumab is more convenient and better tolerated but may be associated with some patients forming anti–alemtuzumab antibodies, particularly those patients who were previously untreated. (Blood. 2004;104:948-955)

© 2004 by The American Society of Hematology

Introduction

Alemtuzumab (CAMPATH-1H, Campath; ILEX Pharmaceuticals, San Antonio, TX) is a humanized immunoglobulin G1 (IgG1) antibody that recognizes the CD52 antigen, a lipid-anchored glycoprotein, on lymphocytes.[1-3] Alemtuzumab is exceptionally lymphoiytic and has been tested as an immunosuppressive agent in transplantation and autoimmune diseases. It is active against a range of lymphoid malignancies and in 2001 was approved for the treatment of B-cell chronic lymphocytic leukemia (B-CLL) in patients who have been treated with alkylating agents and who failed fludarabine therapy. This was based largely on a trial in 93 patients, most of whom had advanced disease with an extremely poor prognosis.[4] After a short dose escalation, alemtuzumab was given intravenously at 30 mg thrice weekly for 4 to 12 weeks. This gave an overall response rate (complete remission [CR] + partial remission [PR]) of 33%. Other trials in chemoresistant CLL reported comparable response rates of 42% and 29% respectively.[5,6]

The dose regimen was developed empirically without the benefit of detailed pharmacodynamic or pharmacokinetic studies. Alemtuzumab often causes substantial first-dose reactions, and although the reactions can now be minimized by appropriate premedication, they are the reason why an initially escalating dose was followed by frequent small doses. This regimen is not the most convenient for patients or physicians. Therefore, the subcutaneous route has been studied in an attempt to reduce side effects and make the treatment more manageable.[7,8]

The different routes of administration can be compared by analysis of the biodistribution of antibody, on the principle that treatments that give comparable blood concentrations should produce similar clinical effects. Some information is available about the pharmacokinetics of intravenous alemtuzumab in patients undergoing stem cell transplantation who have no substantial leukemic burden.[9,10] The terminal half-life was estimated to be approximately 15 to 20 days. However, in patients with advanced CLL, tumor cells could absorb antibody from the circulation.

The humanization of alemtuzumab reduced the risk of antiglobulin responses.[11] However, the antigen binding sites are still potentially immunogenic. The ability to provoke a response depends on the target antigen, the immune status of the patient, the use of immunosuppressive drugs, and other factors, perhaps including the route of administration. There is a suggestion that subcutaneous administration might be more immunogenic than

From the Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom; Millennium Pharmaceuticals, Cambridge, MA; the Department of Haematology, Heartlands Hospital, Birmingham, United Kingdom; the Department of Haematology, Leeds General Infirmary, United Kingdom; the Department of Hematology, Huddinge Hospital, Sweden; the Department of Haematology, Stobhill Hospital, Glasgow, United Kingdom; and the Departments of Hematology and Oncology, Karolinska Hospital, Stockholm, Sweden.

Submitted February 17, 2004; accepted April 4, 2004. Prepublished online as *Blood* First Edition Paper, April 15, 2004; DOI 10.1182/blood-2004-02-0593.

Supported by The EP Abrahams Trust, Oxford, United Kingdom; Millennium Pharmaceuticals Inc, Cambridge, MA; Yorkshire Cancer Research, Yorkshire, United Kingdom; Schering AG, Berlin, Germany; and the Swedish Cancer Society and the Cancer Society in Stockholm, Sweden.

Correspondence: Geoff Hale, Therapeutic Antibody Centre, Old Road, Headington, Oxford, OX3 7JT, United Kingdom; e-mail: geoff.hale@path.ox.ac.uk.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

© 2004 by The American Society of Hematology

Samsung Bioepis Exhibit No. 1002
Page 1148

From www.bloodjournal.org by guest on November 10, 2014. For personal use only.

intravenous administration in patients with rheumatoid arthritis,[12,13] though it is well known that such patients are particularly able to make anti–Ig antibodies.

Here we measured blood concentrations of alemtuzumab and antiglobulin responses against it during 2 different clinical trials. In the first, alemtuzumab was administered intravenously to patients who had previously failed alkylating agents and fludarabine as in the licensed indication. In the second, alemtuzumab was administered subcutaneously to patients who required therapy but had received no prior treatment.[8] We compared the blood concentrations and antiglobulin responses and investigated the possible relationship between alemtuzumab concentrations and clinical responses.

Patients, materials, and methods

Patients

Clinical studies were carried out with the approval of local and, in the United Kingdom, regional ethical committees. All patients gave written informed consent prior to enrolment.

Study design: intravenous study

An open-label phase 2 trial was carried out at 5 centers in the United Kingdom (Aberdeen, Birmingham, Bournemouth, Glasgow, Leeds). Primary objectives included the assessment of safety and efficacy as well as measurement of pharmacokinetics. Patients were eligible if they were at least 18 years old, had confirmed B-CLL requiring treatment, had failed previous treatment with purine analogs, had a World Health Organization (WHO) performance status of 2 or less, and had creatinine and bilirubin concentrations less than twice the upper limit of normal (unless due to disease). Exclusion criteria were active infection, HIV positivity, pregnancy or lactation, history of anaphylaxis to antibodies, less than 3 weeks since chemotherapy or less than 6 weeks since investigational therapy, central nervous system (CNS) involvement or persisting severe pancytopenia, other severe concurrent diseases, secondary malignancy, or mental disorders. Patients were premedicated with paracetamol and chlorpheniramine 30 minutes before the first infusion. Septra (sulfamethoxazole/trimethoprim 800 mg/160 mg twice a day, 3 times per week) and aciclovir (200 mg thrice daily) were given prior to therapy and continued until normal blood lymphocytes returned to the normal range. Allopurinol was given during the first 4 weeks of treatment. Alemtuzumab (M&I Partnership, Cambridge, MA) was diluted in 100 mL of 0.9% saline and administered intravenously over approximately 2 hours. A dose of 3 mg was given on day 1 and if it was well tolerated, a 10-mg dose was given on day 2 and a 30-mg dose on day 3. If severe reactions occurred, the same dose was repeated daily until it was well tolerated and then the dose was escalated. Subsequent 30-mg doses were given thrice weekly for up to 12 weeks, though treatment was occasionally extended if clinically indicated.

Before therapy, a full history, physical examination, and laboratory evaluation were performed, including assessment of lymph node, liver, and spleen size, computed tomographic (CT) scan, Rai staging, blood analysis, bone marrow aspirate, and trephine and immunophenotyping by flow cytometry. Disease parameters were reassessed and blood and bone marrow were reanalyzed by flow cytometry for residual CLL cells every 4 weeks. Therapy was stopped if there was no evidence of CLL or if sequential bone marrow samples showed no fall in the level of CLL. Blood samples were collected weekly to measure alemtuzumab concentrations and antiglobulins. After the last dose, samples were collected daily for 4 days and then weekly for 8 weeks. This was not always possible for logistic or clinical reasons.

During follow-up, patients were assessed monthly for 6 months. Responders were assessed every 6 months thereafter until disease progression. Disease response and toxicity were graded according to the criteria of the National Cancer Institute (NCI).

Study design: subcutaneous study

An open-label phase 2 trial was conducted at 4 clinics at the Karolinska Institute, Stockholm, Sweden.[8] The primary objective was to assess the efficacy of alemtuzumab administered subcutaneously. Patients were eligible if they had a diagnosis of B-CLL, were age 18 to 75, had a WHO performance status of 1 or less, life expectancy at least 12 weeks, required treatment, and had not been treated previously. Other details were comparable to the intravenous study except the treatment regimen. Alemtuzumab was obtained from the Therapeutic Antibody Centre, Oxford, United Kingdom (patients 1-29) or from M&I Partner (patients 30-41). The 2 preparations were similar although the formulations were slightly different and antibody from the Therapeutic Antibody Centre was stored frozen.[14] On day 1, 3 mg alemtuzumab was administered by subcutaneous injection in the thigh. If well tolerated, 10 mg was given on day 3 and 30 mg (in 2 injection sites) was given on day 5. In the event of erythema or edema, the same dose was repeated until well tolerated. After dose escalation, most patients self-administered the antibody. The 30-mg dose was given thrice weekly for up to 18 weeks. If treatment was interrupted for more than 7 days the dose was reinitiated at 3 mg or 10 mg. Therapy was stopped if patients achieved a complete remission (CR) or fulfilled NCI criteria for progressive disease (PD).

Measurement of alemtuzumab in patient serum

Serum samples were collected before doses and at various times afterward and stored at −70°C until analysis by immunofluorescence.[15] Test samples were incubated at 56°C for 30 minutes to inactivate complement then incubated with HUT-78 cells at room temperature for 30 minutes. The cells were washed and resuspended in fluorescein isothiocyanate (FITC)–labeled polyclonal anti–human IgG Fc domain (F-9512; Sigma, Poole, United Kingdom), incubated for 30 minutes, washed and fixed with formaldehyde prior to flow cytometry. Alemtuzumab concentrations were calculated from the median fluorescence by interpolation on a standard curve. The lower limit of quantitation was 0.5 μg/mL (allowing for 2-fold sample dilution), overall precision was plus or minus 13%, and overall accuracy was 109%. There was no interference by normal or patient control sera and no reactivity with $F(ab')_2$ fragments of alemtuzumab.[15]

Measurement of antiglobulin responses

Antiglobulin responses to alemtuzumab were measured in serum samples by sandwich enzyme-linked immunosorbent assay (ELISA) as previously described[11,16] with some modifications. Microtiter plates were coated with alemtuzumab (M&I Partner) and blocked with diluent (phosphate-buffered saline [PBS] containing 2% bovine serum albumin [BSA]). Standards, quality-control samples, and test samples were added, incubated for 1 hour at room temperature, and rinsed 4 times with wash buffer (PBS containing 0.05% Tween 20). Biotin-labeled alemtuzumab was added to each well, incubated for 1 hour, and rinsed as before. The assay was developed with extravidin-peroxidase (E-2886; Sigma) and ortho-phenylenediamine. Color development was measured at 450 nm for 20 minutes by Genesis II software (Thermo Life Sciences, Basingstoke, United Kingdom). The standard was YID13, a monoclonal anti-idiotype specific for alemtuzumab.[16] One U/mL of antiglobulin activity is the signal equivalent to 1 ng/mL of YID13 reference standard. The limit of detection (LOD) and lower limit of quantitation (LLOQ) was 488 U/mL serum (allowing for 2-fold sample dilution), overall precision was plus or minus 14%, and overall accuracy was 94%. There were no false positives and no interference by normal or patient control sera. Positive signals were obtained with serum containing rheumatoid factor or spiked with native CD52 antigen. The assay was reproducible despite variations in reagent concentrations, incubation and washing conditions, blood clotting conditions, and sample storage conditions within the specified ranges. Up to 3-fold enhancement of antiglobulin activity was seen in samples that had been heat-treated or stored for over a month at 4°C. This might be due to aggregation, since aggregates of the control anti-idiotype reagent also gave enhanced activity. Alemtuzumab and anti-alemtuzumab interact with each other to reduce the signal in either assay if both were present in the same sample. Thus, the

From www.bloodjournal.org by guest on November 10, 2014. For personal use only.

antiglobulin assay measures only the concentration of free anti–alemtuzumab antibody, that is, the excess over the levels of alemtuzumab itself. Considering that alemtuzumab concentration was never more than 26 μg/mL, then it might interfere with measurement of antiglobulin responses less than about 26 000 U/mL. However, the antiglobulin levels in a patient who made a clear-cut response exceeded 10^6 U/mL. We report data for all samples measured, both during and, where available, after treatment. For complete assessment of antiglobulin responses, it is always preferable to analyze samples over an appropriate period of time after the end of treatment.

Measurement of minimal residual disease by flow cytometry

Residual CLL cells were quantified by sensitive 4-color flow cytometry; a method more sensitive than consensus-primer polymerase chain reaction (PCR) and more practicable than sequence-specific quantitative PCR.[17] At least 5×10^6 bone marrow leukocytes were incubated with a mixture of fluorescent-labeled antibodies, washed twice, and analyzed using a FACSort (Becton Dickinson, Oxford, United Kingdom) with CELLQuest v3.1 software. The antibodies used were CD45/CD48 (Chemicon, Chandlers Ford, United Kingdom)/CD19/CD3, kappa/lambda/CD19/CD5, CD20 (Beckman Coulter, High Wycombe, United Kingdom)/CD79b (Beckman Coulter)/CD19/CD5, and CD20 (Beckman Coulter)/CD38 (BD Biosciences, Oxford, United Kingdom)/CD19/CD5 conjugated to FITC/phycoerythrin (PE)/PE-Cy5/allophycocyanin (APC), respectively. Reagents without named manufacturer were produced in-house. Between 50 000 and 500 000 cells were analyzed. B cells were identified by setting a region on CD19 versus side scatter (SSC), followed by a forward scatter region, and then ensuring that no $CD3^+$ events fell within the combined gate. CLL cells were discriminated from mature B cells by their stronger expression of CD5 and weaker expression of CD20 and CD79b, and from B progenitors by their weaker expression of CD38. This assay can detect 1 CLL cell in approximately 10^4 to 10^5 leukocytes.

Results

Collection and analysis of samples

Serum samples were obtained from all 30 patients in the intravenous study and 20 of the 41 patients in the subcutaneous study, almost exclusively from a single clinic (Karolinska Hospital). Samples were tested in duplicate and the mean concentration was calculated. If the mean was less than 0.5 μg/mL (LLOQ), a level of less than 0.5 μg/mL was recorded. If either result was more than 20 μg/mL or a measurement was technically faulty, then the test was repeated, using a higher dilution if appropriate, and the repeat results were used.

Optimal time for collection of peak samples following intravenous administration

A preliminary experiment was carried out to identify the time following intravenous administration when the peak antibody concentration might be found. Samples were collected from 3 patients at 15 minutes, 30 minutes, and 60 minutes after the end of one dose each week for 4 to 7 weeks. The peak sample occurred at 15 minutes, significantly more often (21 times) than at 30 minutes (6 times) or 60 minutes (2 times) ($P < .01$; paired Wilcoxon signed rank test). Therefore, for subsequent patients a single sample was collected at 15 minutes to determine the peak concentration.

Serum concentrations of alemtuzumab during intravenous treatment

A total of 1561 patient samples were tested. Terminal samples (after the last dose) were not available for some patients, and in a few cases only a small number of terminal samples were available.

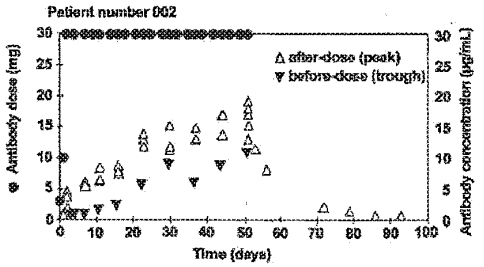


Figure 1. Example of alemtuzumab concentrations in patient treated with intravenous antibody. After an initial dose escalation, 30 mg of alemtuzumab was administered 3 times a week for 8 weeks (●). Serum samples were taken before (Δ) and after (▼) the dose once a week. This patient made a good clinical response to the therapy.

All samples were tested in duplicate; the overall mean coefficient of variation (CV) was 10%. Many of the early samples and many trough samples were below the limit of quantitation (< 0.5 μg/mL). Five samples gave anomalous results, substantially diverging from other results for the same patients. These could be explained by accidental mix-ups at the clinical centers and were omitted from analysis. An example of the doses and blood concentrations throughout the course of treatment for one patient is shown in Figure 1.

In contrast to patients who had received transplants,[9,10] there were wide variations in alemtuzumab concentrations between different patients with CLL. The highest peak ranged from 2.8 μg/mL to 26.4 μg/mL (mean 10.7 μg/mL). The highest trough ranged from less than 0.5 μg/mL to 18.3 μg/mL (mean 5.4 μg/mL). The cumulative dose before the trough concentration reached 1.0 μg/mL ranged from 13 mg to 316 mg (mean 90 mg; Table 1). In 9 of 21 evaluable patients, the highest trough concentration was measured just before the last dose, indicating that a steady state had not been reached. Total cumulative doses varied between patients, but did not correlate with the final antibody concentrations (Table 1).

Comparison of the starting lymphocyte count with the peak concentration following the first 30-mg infusion showed a modest negative correlation, that is, the higher the initial lymphocyte count, the lower tended to be the peak concentration of alemtuzumab (Figure 2).

In Figure 3 the highest trough concentration for each patient is plotted against the response measured by NCI criteria for 27 patients where the clinical response was reported within 4 months of the end of treatment. There was a significant increase in trough

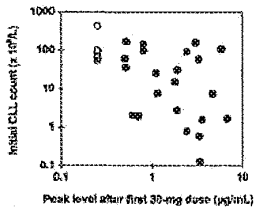


Figure 2. Relationship between initial CLL count and alemtuzumab blood concentration: intravenous study. The numbers of CLL cells in the blood directly before intravenous therapy are plotted against the peak concentration of alemtuzumab measured directly after the first 30-mg dose (●). In 8 patients the concentration was below the limit of quantitation, and these have all been plotted at an arbitrary level of 0.25 μg/mL (○). Pearson correlation coefficient for log (initial CLL count) versus log (alemtuzumab concentration) was −0.44, $P = .01$.

Samsung Bioepis Exhibit No. 1002
Page 1150

From www.bloodjournal.org by guest on November 10, 2014. For personal use only.

Table 1. Alemtuzumab dose and serum concentrations: intravenous study

Patient no.	Cumulative dose, mg	Number of samples	Terminal samples	Dose to reach 1 μg/mL	Maximum peak, μg/mL	Maximum trough, μg/mL	Before last dose, μg/mL	After last dose, μg/mL
001	1026	80	12	143	16.3	8.8	7.7	11.7
002	673	58	12	13	19.1	13.0	13.0	19.1
003	1106	91	23	43	6.7	1.1	0.9	5.9
004	836	54	0	103	26.4	18.3	ND	ND
005	1064	69	7	46	15.3	6.2	6.2	16.3
006	736	55	8	18	3.5	3.7	2.5	8.5
007	1093	75	11	133	19.8	11.9	11.9	15.4
008	291	19	3	111	5.6	0.7	0.7	0.7
009	1026	73	12	53	13.3	7.7	7.7	12.2
010	179	21	0	46	4.0	0.8	ND	ND
011	1076	73	11	133	8.3	1.9	1.9	8.8
012	473	26	0	53	4.4	0.2	ND	ND
013	846	60	13	163	8.0	0.5	< 0.5	2.9
014	436	31	0	46	12.5	0.9	ND	ND
015	2053	89	0	43	6.4	2.8	ND	ND
016	1016	58	11	103	9.6	4.9	4.6	9.0
017	1093	55	8	133	8.6	1.7	1.2	6.0
018	733	40	1	43	8.2	2.6	ND	ND
019	493	27	1	73	18.9	14.8	ND	ND
020	1083	65	12	43	17.7	9.6	7.7	12.8
021	583	32	0	163	4.0	1.1	ND	ND
022	1003	45	5	43	7.1	1.0	0.6	6.3
023	1061	47	6	43	26.0	17.0	ND	24.1
024	1126	57	12	316	6.0	2.6	2.0	5.5
025	1093	54	11	73	6.8	5.2	3.1	6.7
026	869	57	13	133	5.4	1.5	1.4	4.7
027	1066	45	10	73	2.8	0.9	0.5	2.5
028	673	42	13	43	6.5	4.0	4.0	6.9
029	416	40	10	133	7.6	3.3	3.3	7.6
030	763	35	5	133	12.2	3.7	3.7	12.2
Minimum	179	19	0	13	2.8	< 0.5	< 0.5	0.7
Mean	873	52	6	90.4	10.7	5.4	4.0	9.4
Maximum	2053	91	23	316	26.4	18.3	13.0	24.1

ND indicates not determined.

concentrations with increasing clinical response ($P = .006$, Kruskal-Wallis test). There was an even more striking correlation between trough concentrations and the attainment of minimal residual disease (MRD) negativity in 7 patients (filled symbols) compared with the 17 patients who still had residual CLL cells in the bone marrow at the end of treatment as measured by 4-color flow cytometry ($P < .0001$, Kruskal-Wallis test)

In Figure 4 the mean trough concentration is plotted throughout the treatment for 2 groups of patients: (1) 8 responders who reached MRD less than 0.4%, and (2) 22 others. The mean trough concentration was significantly higher in the responders throughout the whole course of treatment from week 2 to week 11 ($P < .02$ at each time point, Kruskal-Wallis test).

Clearance of alemtuzumab following the last dose showed a similar correlation with residual disease. In patients with undetectable CLL cells, there was a single clearance phase characterized by

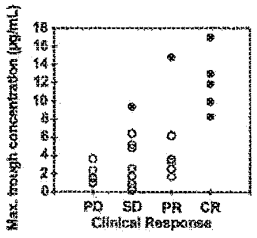


Figure 3. Relationship between maximum trough concentrations of alemtuzumab and clinical response: intravenous study. The highest trough concentration of alemtuzumab (which generally occurred following the penultimate or last dose) is plotted against the clinical response at the end of intravenous treatment as determined by NCI criteria. PD indicates progressive disease; SD, stable disease; PR, partial remission; CR, complete remission. A better clinical outcome was significantly associated with higher alemtuzumab concentrations ($P = .006$, Kruskal-Wallis test). There was an even stronger correlation between high trough concentrations (> 8 μg/mL) and good responses measured by less than 0.1% CLL cells in the bone marrow (●; $P < .0001$). Three patients who died before the end of the planned treatment course are not included in this figure. Of those patients, 2 had low alemtuzumab concentrations (≤ 1.1 μg/mL) and progressive disease at the time of death. The third had a high antibody concentration (18.3 μg/mL) and low levels of residual disease (0.21%). Each symbol represents a single patient; ● indicates a patient with no detectable MRD; ○, a patient with detectable MRD.

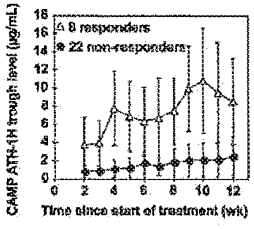


Figure 4. Mean trough concentrations of alemtuzumab during intravenous therapy. The mean trough concentrations during treatment (measured 48 hours after a dose, once a week) are plotted, with standard deviations, for 8 patients who ultimately reached less than 0.4% CLL cells in the bone marrow (Δ) and compared with 22 patients who still had residual CLL cells at the end of treatment (●).

Samsung Bioepis Exhibit No. 1002
Page 1151

From www.bloodjournal.org by guest on November 10, 2014. For personal use only.

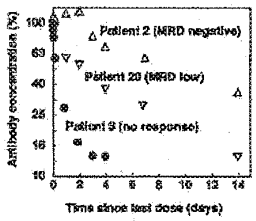


Figure 5. Clearance of alemtuzumab in selected patients treated intravenously. In patients with minimal residual disease (by flow cytometry) at the end of therapy, clearance was slow and single phase (△). Patients who still had a small amount of CLL cells showed biphasic clearance (▽) and patients who still had bulky disease cleared the antibody rapidly (⊛). However, in each case the terminal half-life was roughly similar.

a relatively long half-life. In patients who still had a substantial tumor burden, most of the antibody was rapidly cleared from the blood. Patients with low levels of residual disease showed an intermediate pattern. An example of each is shown in Figure 5.

Sixteen patients contributed informative terminal samples after the last 30-mg dose. From these data the following mean pharmacokinetic parameters were calculated (ILEX Oncology, unpublished data, September 2001). The apparent steady-state volume of distribution (V_{SS}) was 0.185 L/kg and the apparent volume of distribution during the terminal phase (VZ) was 0.252 L/kg. These are larger than for other monoclonal antibodies in humans but are consistent with the notion that alemtuzumab distributes through the plasma compartment and a substantial extravascular lymphocyte compartment. The mean terminal phase half-life ($t_{1/2,\ z}$) was 6.1 days, which is consistent with previous measurements for alemtuzumab and other chimeric antibodies in humans.

Serum concentrations of alemtuzumab during subcutaneous treatment

This study was started in 1998 using alemtuzumab manufactured at the Therapeutic Antibody Centre. During June 2000, commercially produced material became available and was subsequently used. No obvious differences were observed in the clinical responses. A total of 542 patient samples were received and tested. Two were mislabeled and are not included here. The overall mean CV was 8%. Assessment of pharmacokinetics in 7 patients was inadequate due to the limited number or inappropriate timing of samples.

Patient no. 8036 (who started with alemtuzumab from the Therapeutic Antibody Centre and was switched to commercial alemtuzumab) never achieved concentrations above the LLOQ. This patient had rapidly made a strong antiglobulin response to the alemtuzumab, which probably neutralized all of the administered antibody ("Antiglobulins following subcutaneous treatment"). The patient never showed any clearance of lymphocytes but showed continued reactions at the injection site. Because of this, the patient was switched to intravenous administration after 6 weeks.

There were some changes from the dose regimen due to clinical reactions. There were 2 patients who received lower doses throughout (10 mg and 20 mg) and 2 (including patient no. 8036, above) who were switched to intravenous dosing. The dose escalation rate was often slower than originally planned, typically taking 1 to 2 weeks to reach 30 mg. After this, the planned dose was usually maintained but only 1 of the 21 patients analyzed received the maximum planned dose of 1573 mg. The mean total dose was 1249 mg and the lowest dose was 712 mg.

The highest concentrations ranged from 0.6 μg/mL to 24.8 μg/mL and the mean (5.4 μg/mL) was the same as in the intravenous study (Table 2). However, the cumulative dose before the concentration reached 1.0 μg/mL ranged from 146 mg to 1106 mg (mean 551 mg), which was substantially more than in the intravenous study. An example of the time course in one patient illustrating this delay is shown in Figure 6.

Inadequate samples were available for analysis of terminal phase pharmacokinetics. No correlation could be demonstrated between alemtuzumab concentrations and clinical responses because nearly all of the patients (18/20) were responders (CR or PR), and levels of MRD were not reported.

Antiglobulins following intravenous treatment

A total of 519 serum samples from 30 patients treated with intravenous alemtuzumab were tested for antiglobulins. These included all samples taken more than 24 hours after the last dose and representative pre-dose samples during treatment. All of them were below the limit of detection (488 U/mL). In 12 patients there were no or very few samples available after the end of treatment, and in the other 18 patients samples were only available between 2 and 6 weeks after the last dose. Therefore, we cannot completely exclude the possibility of delayed responses.

Antiglobulins following subcutaneous treatment

A total of 281 samples were tested from 21 patients (one patient had adequate late samples for antiglobulin measurement, but none suitable for pharmacokinetic analysis). These included all samples taken after the last dose and representative samples taken during treatment. There were 4 patients who had no or few samples after the end of treatment but 17 who had adequate posttreatment samples. All samples except those described below were below the LOD.

One patient (no. 8027) had a single sample taken at day 238 (114 days after the last dose) that gave a weak signal just above the LOD. All previous and subsequent samples were less than the LOD. The patient made a good clinical response to therapy, achieving a CR, and no relevant adverse effects were noted. It is possible that the patient made a very weak, transient response to the alemtuzumab, but we doubt that it could have been clinically significant.

One patient (no. 8036) made a very high-titer response, reaching a peak of approximately 3.7×10^6 U/mL 24 days after the last dose of alemtuzumab (Figure 7). This antiglobulin bound strongly to CAMPATH-1G, the parental rat antibody from which alemtuzumab was humanized, but did not bind other human IgG1

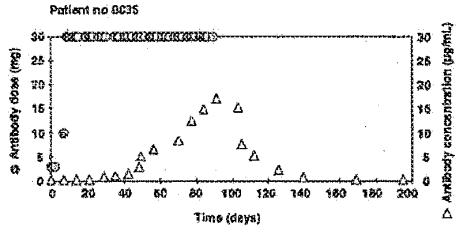


Figure 6. Example of alemtuzumab concentrations in a patient treated with subcutaneous antibody. After an initial dose escalation, 30 mg of alemtuzumab was administered 3 times a week for 12 weeks (◎). Serum samples were taken before the dose, once a week (△). This patient made a good clinical response to the therapy and achieved relatively high concentrations of antibody.

Samsung Bioepis Exhibit No. 1002
Page 1152

From www.bloodjournal.org by guest on November 10, 2014. For personal use only.

Table 2. Alemtuzumab dose and serum concentrations: subcutaneous study

Patient no.	Cumulative dose, mg	No. of samples	Above LOQ	Terminal samples	Dose to reach 1 μg/mL	Max concentration, μg/mL	After last dose, μg/mL
8010	1355	23	2	0	728	1.3	ND
8015	1506	28	1	0	> 156	0.6	ND
8016	1573	23	3	0	673	1.5	ND
8017	1213	20	2	0	193	0.9	ND
8021	958	29	12	3	353	5.3	8.1
8026	1379	23	5	0	959	1.4	ND
8027	1269	43	8	1	539	9.0	5.8
8028	1363	37	13	3	403	5.8	5.8
8029	1433	34	9	1	503	7.3	7.3
8030	1553	45	16	1	473	4.3	4.2
8031	712	27	2	1	712	1.4	1.4
8032	725	17	2	0	393	2.5	2.5
8033	1302*	28	10	0	542	3.4	3.9
8034	1412	24	16	4	268	6.3	6.8
8035	1039	24	15	6	406	17.1	17.1
8036	819*	21	0	0	> 819	0.5	< 0.5
8038	1485	19	5	3	1108	2.6	2.6
8039	1526	19	10	0	595	4.4	ND
8040	1496	26	19	7	146	24.8	24.8
8041	852	23	6	0	462	3.5	3.5
Minimum	712	17	0	0	146	0.6	< 0.5
Mean	1249	27	8	1.5	551	5.4	6.4
Maximum	1573	45	19	7	1108	24.8	24.8

*Some doses for patients no. 8033 and no. 8036 were given intravenously due to infusion site reactions during the subcutaneous administration.

monoclonal antibodies. Therefore, essentially all of the response was directed against the alemtuzumab idiotype. No free alemtuzumab could be detected throughout treatment and there was no significant depletion of tumor cells during therapy. This patient developed a pronounced injection site reaction including erythema, edema, and slight local pain but otherwise no marked general symptoms. As a result, the patient did not achieve the 30-mg dose level during 6 weeks of subcutaneous dosing and alemtuzumab was subsequently administered intravenously. Due to nonresponsiveness, the patient was subsequently treated with fludarabine and went into a long-lasting partial remission. He was retested 18 months after the end of alemtuzumab treatment (continuing in PR) and still had a high-titer antiglobulin (approx 1.8×10^5 U/mL). There was a very low anti-alemtuzumab signal in one of 2 samples taken before therapy. This patient might have had a natural anti-IgG (rheumatoid factor) prior to therapy and perhaps was predisposed to make an antiglobulin response.

A search was made for samples from other patients who had received subcutaneous alemtuzumab. Samples were collected between 4 to 50 months after the end of treatment from an additional 9 patients of the original subcutaneous study plus 2 others who received subcutaneous alemtuzumab as part of a compassionate program. A substantial response was detected in patient no. 8020 of approximately 4800 U/mL at 32 months after the end of treatment. Like the response in patient no. 8036, this response cross-reacted on the parental rat antibody but not on other human antibodies, showing it to be essentially anti-idiotype. The patient had not responded to the therapy, but was reported to have prolonged skin reactions to the infusions. A very weak response was detected in one other patient of 541 U/mL, barely above the LOD. Unlike the other 2 patients, this serum cross-reacted with other human IgG but not with CAMPATH-1G. We consider that it is a nonspecific rheumatoid-like factor and probably had no clinical significance with respect to alemtuzumab.

In total therefore, of 32 patients treated with subcutaneous alemtuzumab (30 who were previously untreated with any anti-CLL agent), 2 produced a significant antiglobulin response which persisted at potentially neutralizing concentrations for more than a year. Both of these patients had received alemtuzumab manufactured at the Therapeutic Antibody Centre, as did most of the patients in this study, although patient no. 8036 had been switched to the commercial product after 3 weeks.

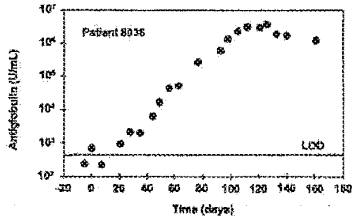


Figure 7. Antiglobulin response to alemtuzumab following subcutaneous treatment. This patient received subcutaneous alemtuzumab at doses up to 10 mg until day 37 (cumulative dose, 89 mg) and subsequently received 30 mg 3 times a week intravenously until day 80, followed by 30 mg 3 times a week subcutaneously until day 102. Samples were taken once a week to measure antiglobulin responses. One U/mL is equivalent to 1 ng/mL of a standard monoclonal anti-idiotype. The horizontal line indicates the limit of detection (LOD).

Discussion

Alemtuzumab is extensively used for patients with B-CLL who have failed treatment with alkylating agents and purine analogues and investigators are exploring its use in other disease settings. To enable safe and effective use it is essential to understand alemtuzumab's pharmacokinetics and biodistribution as well as the potential for anti-idiotype responses, which all may differ according to disease, patient status, and route of administration. We have started to address these issues by comparing blood concentrations of

From www.bloodjournal.org by guest on November 10, 2014. For personal use only.

alemtuzumab and antiglobulin responses in 2 different studies for treatment of CLL, namely intravenous treatment of chemotherapy-resistant disease and first-line subcutaneous treatment. The studies were initiated independently and had different objectives; nevertheless the pharmacologic results are instructive.

Alemtuzumab may cause substantial "first dose" reactions as a result of cytokine release when it is given intravenously.[5,18,19] Therefore, the standard treatment regimen involves dose escalation and frequent, relatively small doses, though it has not been formally established whether the reactions are strictly dose related. A treatment course for chemotherapy-resistant CLL consists of approximately one gram of the antibody. However, a cumulative dose of 40 mg to 100 mg is highly immunosuppressive and clinically effective in patients without bulky tumors, for example those treated for prevention of transplant rejection,[11,20] multiple sclerosis,[21] and other autoimmune diseases,[22-24] as well as to eradicate detectable minimal residual disease in CLL.[25] The biodistribution of alemtuzumab is likely to depend on the bulk of tumor cells, possibly more so than other monoclonals because CD52 is such a highly expressed antigen.

There are approximately 5×10^5 binding sites for alemtuzumab on a normal lymphocyte.[26,27] There are about 10^{12} lymphocytes in a healthy adult.[28] At least 125 mg of alemtuzumab is therefore required to saturate all of the CD52 sites. CLL cells express a similar amount of CD52 antigen to normal lymphocytes[27] but their total number may be 10 times greater. Therefore, more than 1 g of alemtuzumab could be required to saturate all the receptors in some patients, especially considering that the antibody has a relatively low affinity and that there will be a significant clearance during a 12-week course of treatment. Hence, it is not surprising that the biodistribution and clearance of antibody during the treatment of patients with CLL is dominated by the tumor burden and cannot be fitted to a simple pharmacokinetic model.

There was a strong correlation between the concentrations of antibody throughout intravenous treatment and the ultimate clinical response, or the achievement of minimal residual disease (MRD) negativity in the bone marrow by flow cytometry. It has recently been reported that survival correlates better with MRD than with the standard NCI criteria for clinical responses.[29] Without a good measure for the initial total tumor burden it is difficult to distinguish cause and effect. One possibility is that patients with high tumor burden were treated with inadequate amounts of antibody to achieve sufficient receptor site saturation and see a clinical effect. In any event, it appears that measurements of alemtuzumab concentrations after 2 to 4 weeks of treatment might provide an indicator of the ultimate outcome and guide the physician whether to continue, stop, or adjust the dose of antibody. Most of the antibody was cleared within a few days of the final dose in the nonresponders. However, the half-life at the end of treatment in the responders was comparable with those previously reported for patients without bulky tumors.

Accumulation of alemtuzumab in the blood was significantly slower in the subcutaneous study and it took on average about 6 weeks longer to reach 1.0 μg/mL (an arbitrary threshold known to be potentially lympholytic). This could be a slight overestimate because samples were not always collected every week. Nevertheless, the eventual maximum trough concentrations were very similar in both studies. We cannot tell whether the slower accumulation of antibody in the blood was due to less favorable biodistribution, more effective binding to tumor cells, or the slower dose escalation used in the subcutaneous study compared with that used with intravenously treated patients. It should be possible to resolve this by studies of alemtuzumab given subcutaneously to patients not suffering from malignancies (ie, without excess $CD52^+$ cells).

Because clinical responses were much more frequent (19% CR and 68% PR),[8] we could not detect a significant correlation between blood concentrations and clinical responses in the subcutaneous study. With only 2 nonresponders in the group of patients we analyzed, the sample was too small to discern any relationship.

Clear-cut results were obtained from the measurements of antiglobulin responses (antihuman antibody [HAHA]). There were no detectable responses at all in the intravenous study (heavily pretreated patients) and only one patient in the subcutaneous study (previously untreated patients) made a strong response, reaching a peak of approximately 3.7×10^6 U/mL 3 weeks after the final dose. This was equivalent to 3.7 mg/mL of the monoclonal anti-idiotype standard and represented a substantial proportion of the total Ig in the patient. Similar titers were previously reported during treatment of rheumatoid arthritis patients with subcutaneous alemtuzumab[13] when all of the patients (10/10) made a positive response. After screening 11 other patients we found one with a significant antiglobulin titer of 4800 U/mL. Considering that this sample was taken 32 months after the last treatment with alemtuzumab, it seems likely that this patient also had made a very strong response.

Previous studies on antiglobulin responses to alemtuzumab have given widely different results. In 12 patients treated for kidney transplant rejection no antiglobulins were detected, in contrast to 15 of 17 patients treated with the parental rat antibody CAMPATH-1G.[11] In 167 patients treated for chemotherapy-resistant lymphoid malignancies there were only 3 weak or borderline responses. However, out of 115 patients treated for rheumatoid arthritis, there were 59 positive responses (ILEX Pharmaceuticals, unpublished data on file, July 2003), some of high titer similar to that reported here for patient no. 8036.[13] Many factors could affect the immunogenicity of alemtuzumab, including the dose and length of treatment, the underlying disease, prior exposure to chemotherapy, concomitant immunosuppressive drugs, and possibly the route of administration or the formulation of the drug. Subcutaneous administration might allow more effective antigen presentation, for example, by Langerhans cells and with less "excess" antibody circulating to provide tolerogenic signals, the balance might be tipped toward an immune response. From our present study we cannot say whether subcutaneous administration in CLL is more immunogenic than intravenous administration since there were other differences between the 2 groups, most notably the subcutaneous group had received no prior chemotherapy. Even if the 2 groups had been comparable, the difference in responses (0/30 vs 2/32) did not reach statistical significance. This issue needs to be investigated in larger numbers of patients. Both patients who made an antiglobulin response had been treated with alemtuzumab manufactured at the Therapeutic Antibody Centre. We are aware that the manufacture, formulation, or storage of biologics might affect their immunogenicity, but so far we have no evidence to suggest that there is a major difference between the academic or commercial source since antiglobulin responses have been reported in other patients treated with alemtuzumab from either source. The 2 patients (no. 8020 and no. 8036) who produced anti–alemtuzumab antibody on subcutaneous treatment, unlike the other patients, did not show significant reductions in lymphocyte counts but had marked local skin reactions which did not diminish with continued therapy. An antiglobulin response should be considered when there is no drop in the blood lymphocyte count and/or there are persistent injection site reactions.

Samsung Bioepis Exhibit No. 1002
Page 1154

From www.bloodjournal.org by guest on November 10, 2014. For personal use only.

Alemtuzumab provides a useful therapeutic option for patients with CLL. It has excellent activity as first-line treatment and can give good responses in patients who are unresponsive to conventional chemotherapy. The optimal dose route and regimen are not yet known. However, the subcutaneous route can deliver concentrations of antibody comparable to the original intravenous route. The dominant factor influencing biodistribution and pharmacokinetics appears to be the tumor burden. With intravenous dosing, serum concentrations of alemtuzumab can provide an early prediction of the ultimate response and might be useful to guide dose adjustments to improve responses or reduce side effects. Strong anti-idiotype responses may occur in a small minority of patients. Although not associated with serious adverse effects, such responses neutralize the therapeutic benefit and therefore patients with a known antiglobulin response should not be further treated with this monoclonal antibody.

Acknowledgments

We are very grateful to the staff of the Therapeutic Antibody Centre, Oxford, United Kingdom, for the manufacture and supply of some of the alemtuzumab used in this study. We thank Pete Bonate, Ian Clements, and the team at ILEX Oncology for constructive comments and for sharing unpublished data.

References

1. Hale G, Waldmann H. From laboratory to clinic: the story of CAMPATH-1. In: George AJT, Urch C, eds. Antibodies in the Clinic: Methods in Molecular Medicine, vol 40: Diagnostic and Therapeutic Antibodies. Humana Press, NJ; 2000:243-266.
2. Hale G. CD52. J Biol Regul Homeost Agents. 2002;15:386-391.
3. Dumont FJ. CAMPATH (alemtuzumab) for the treatment of chronic lymphocytic leukemia and beyond. Expert Rev Anticancer Ther. 2002;2:23-25.
4. Keating MJ, Flinn I, Jain V, et al. Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. Blood. 2002;99:3554-3561.
5. Osterborg A, Dyer MJS, Bunjes D, et al. Phase II multicenter study of human CD52 antibody in previously treated chronic lymphocytic leukemia. European Study Group of CAMPATH-1H treatment in chronic lymphocytic leukemia. J Clin Oncol. 1997;15:1567-1574.
6. Rai K, Freter CE, Mercier RJ, et al. Alemtuzumab in previously treated chronic lymphocytic leukemia patients who also had received fludarabine. J Clin Oncol. 2002;20:3891-3897.
7. Osterborg A, Fassas AS, Anagnostopoulos A, Dyer MJD, Catovsky D, Mellstedt H. Humanized CD52 monoclonal antibody Campath-1H as first line treatment in chronic lymphocytic leukaemia. Br J Haematol. 1996;93:151-153.
8. Lundin J, Kimby E, Bjorkholm M, et al. Phase II trial of subcutaneous anti-CD52 monoclonal antibody alemtuzumab (Campath-1H) as first-line treatment for patients with B-cell chronic lymphocytic leukemia (B-CLL). Blood. 2002;100:768-773.
9. Rebello P, Cwynarski K, Varughese M, Eades A, Apperley J, Hale G. Pharmacokinetics of CAMPATH-1H in BMT patients. Cytotherapy. 2001;3:261-267.
10. Morris E, Rebello, P, Thomson K, et al. Pharmacokinetics of alemtuzumab used for in vivo and in vitro T-cell depletion in allogeneic transplantations: relevance for early adoptive immunotherapy and infectious complications. Blood. 2003;102:404-406.
11. Rebello P, Hale G, Friend PJ, Cobbold SP, Waldmann H. Anti-globulin responses to rat and humanised CAMPATH-1 monoclonal antibody used to treat transplant rejection. Transplantation. 1999;68:1417-1420.
12. Isaacs JD, Watts RA, Hazleman BL, et al. Humanized monoclonal antibody therapy for rheumatoid arthritis. Lancet. 1992;340:748-752.
13. Schnitzer TJ, Yocum DE, Michalska M, et al. Subcutaneous administration of CAMPATH-1H: clinical and biological outcomes. J Rheumatol. 1997; 24:1031-1036.
14. Phillips J, Drumm A, Harrison P, et al. Manufacture and quality control of CAMPATH-1 antibodies for clinical trials. Cytotherapy. 2001;3:233-242.
15. Rebello P, Hale G. Pharmacokinetics of CAMPATH-1H: assay development and validation. J Immunol Meth. 2002;260:285-302.
16. Cobbold SP, Rebello PRUB, Davie Hff, Friend PJ, Clark MR. A simple method for measuring patient anti-globulin responses against isotypic or idiotypic determinants. J Immunol Meth. 1990;127: 19-24.
17. Rawstron AC, Kennedy B, Evans PAS, et al. Quantitation of minimal disease levels in chronic lymphocytic leukemia using a sensitive flow cytometry assay improves the prediction of outcome and can be used to optimise therapy. Blood. 2001;98:29-35.
18. Moreau T, Coles A, Wing M, et al. Transient increase in symptoms associated with cytokine release in patients with multiple sclerosis. Brain. 1996;119:225-237.
19. Wing MG, Moreau T, Greenwood J, et al. Mechanism of first-dose cytokine-release syndrome by CAMPATH-1H: involvement of CD16 (FcgRIII) and CD11a/CD18 (LFA-1) on NK cells. J Clin Invest. 1996;98:2819-2826.
20. Calne R, Moffatt SD, Friend PJ, et al. CAMPATH-1H allows low-dose cyclosporin monotherapy in 31 cadaveric renal allograft recipients. Transplantation. 1998;66:1613-1616.
21. Coles AJ, Wing MG, Molyneux P, et al. Monoclonal antibody treatment exposes three mechanisms underlying the clinical course of multiple sclerosis. Annal Neurol. 1999;46:296-304.
22. Lockwood CM, Thiru S, Isaacs JD, Hale G, Waldmann H. Humanised monoclonal antibody treatment for intractable systemic vasculitis. Lancet. 1993;341:1620-1622.
23. Lockwood CM, Thiru S, Stewart S, et al. Treatment of refractory Wegener's granulomatosis with humanised monoclonal antibodies. Q J Med. 1996;89:903-912.
24. Willis F, Marsh J, Bevan D, et al. The effect of treatment with Campath-1H in patients with autoimmune cytopenias. Brit J Haematol. 2001;114: 891-898.
25. Montillo M, Cafro AM, Tedeschi A, et al. Safety and efficacy of subcutaneous Campath-1H for treating residual disease in patients with chronic lymphocytic leukemia responding to fludarabine. Haematologica. 2002;87:695-700.
26. Bindon CI, Hale G, Waldmann H. Importance of antigen specificity for complement mediated lysis by monoclonal antibodies. Eur J Immunol. 1988; 18:1507-1514.
27. Rossmann ED, Lundin J, Lenkei R, Mellstedt H, Osterborg A. Variability in B-cell antigen expression: implications for the treatment of B-cell lymphomas and leukemias with monoclonal antibodies. Hematol J. 2001;2:300-306.
28. Series HE, Remmers AR, Fish JC, et al. Depletion of lymphocytes for the protection of renal allografts. Arch Intern Med. 1970;125:443-450.
29. Moreton P, Kennedy DB, Rawstron AC, Hillmen P. Achieving a MRD negative response after alemtuzumab for CLL is the best predictor for prolonged survival [abstract]. Blood. 2003;102: 5290a.

Samsung Bioepis Exhibit No. 1002
Page 1155

Pergamon
PII: S0161-5890(97)00042-4

Molecular Immunology, Vol. 34, No. 6, pp. 441–452, 1997
© 1997 Elsevier Science Ltd. All rights reserved
Printed in Great Britain
0161-5890/97 $17.00 + 0.00

HUMANIZED PORCINE VCAM-SPECIFIC MONOCLONAL ANTIBODIES WITH CHIMERIC IgG2/G4 CONSTANT REGIONS BLOCK HUMAN LEUKOCYTE BINDING TO PORCINE ENDOTHELIAL CELLS

JOHN P. MUELLER,*MICHELLE A. GIANNONI, SANDRA L. HARTMAN, EILEEN A. ELLIOTT, STEPHEN P. SQUINTO, LOUIS A. MATIS and MARK J. EVANS

Alexion Pharmaceuticals Inc., Departments of Immunobiology and Molecular Development, 25 Science Park Box 15, New Haven, CT 06511, U.S.A.

(*First received* 21 *January* 1997; *accepted in revised form* 1 *April* 1997)

Abstract—Discordant xenografts surviving the initial hyperacute rejection phase may be subject to cellular rejection processes mediated by infiltrating leukocytes including T cells, NK cells and monocytes. The stable adhesion of these cell types to endothelial cells is due to the molecular interaction of the integrins VLA-4 and LFA-1 with their ligands vascular cell adhesion molecule (VCAM) and ICAM-1 present on the endothelial cells. Human VLA-4 binds to porcine VCAM, and blocking mAbs specific for porcine VCAM have been developed. We have localized the epitope of the anti-porcine VCAM blocking mAbs 2A2 and 3F4 to domains 1 and 2, respectively. Humanized antibodies (IgG4 isotype) were constructed from these anti-porcine VCAM antibodies and demonstrated to inhibit adhesion of Ramos, Jurkat and YT cells, as well as purified resting and activated human T cells, to porcine aortic endothelial cells (PAEC). These cell types express both LFA-1 as well as VLA-4, suggesting blockade of human VLA-4 interaction with porcine VCAM may alone be sufficient to significantly impair adhesion of human leukocytes to porcine endothelial cells. The chimeric anti-porcine VCAM (pVCAM) HuG4 antibodies promoted increased adhesion of Fc receptor (FcR) positive cells such as U937 monocytic cells to PAEC. In contrast, chimeric anti-porcine VCAM antibodies created using the CH1 and hinge region from human IgG2 and the CH2 and CH3 regions from human IgG4 (HuG2/G4 antibodies) inhibited binding of FcR positive cells to PAEC. These chimeric anti-pVCAM antibodies should allow delineation of the *in vivo* role of VLA-4/VCAM interaction in porcine-to-primate xenotransplants. Further, the design of the HuG2/G4 antibodies should render them efficacious in multiple settings requiring elimination of FcR binding. © 1997 Elsevier Science Ltd.

Key words: adhesion molecules, antibodies, Fc receptors, inflammation, xenotransplantation.

INTRODUCTION

Although much progress has been made in elucidating the molecular mechanisms of hyperacute rejection of xenotransplants (reviewed in Somerville and D'Apice, 1993), the mechanisms of cell mediated rejection of xenografts remain unclear. The cellular infiltrate observed 3 days following transplantation of guinea pig hearts into complement (C)-depleted rats is comprised predominantly of macrophages and natural killer cells, with low numbers of T lymphocytes present (Blakely *et al.*, 1994). Similarly, the cellular infiltrate present 6 days after transplantation of porcine fetal islet clusters into rats is comprised predominantly of macrophages with low numbers of T lymphocytes present primarily at the periphery of the graft (Wallgren *et al.*, 1995). *In vitro* porcine aortic endothelial cells (PAEC) as well as antigen presenting peripheral blood cells can directly activate human CD4+ and CD8+ T lymphocytes (Rollins *et al.*, 1994*a*; Murray *et al.*, 1994; Yamada *et al.*, 1995). Thus the initial phases of cellular rejection of xenotransplants are likely to involve binding and activation of multiple types of leukocytes including not only T lymphocytes but also natural killer cells and monocytes.

The stable adhesion of lymphocytes, natural killer cells, and monocytes to endothelial cells is mediated predominantly by the interaction of the integrins LFA-1 (CD11a/CD18) and VLA-4 (CD49d/CD29) with their cognate ligands ICAM-1 and VCAM-1 on endothelial cells (Allavena *et al.*, 1991; Jonjic *et al.*, 1992). For both monocytes (Luscinskas *et al.*, 1994) and T lymphocytes (Alon *et al.*, 1995; Luscinskas *et al.*, 1995) the binding of VLA-4 to VCAM is an essential component of the *in vitro* adhesive interactions required for stable arrest under

*Author to whom correspondence should be addressed. Tel: 203-776-1790; Fax: 203-772-3655; E-mail: muellerj@alxn.com.

Samsung Bioepis Exhibit No. 1002
Page 1156

conditions of flow. *In vitro*, the transmigration of NK cells and monocytes across human umbilical vein endothelial cell (HUVEC) monolayers also utilizes both LFA-1/ICAM-1- and VLA-4/VCAM-dependent pathways (Bianchi *et al.*, 1993; Meershaert and Furie, 1994; Chuluyan *et al.*, 1995). *In vivo*, the interaction of LFA-1 with ICAM-1 may not be completely essential for extravasation of lymphocytes or monocytes since both of these cell types are present in inflammatory infiltrates of leukocyte adhesion deficiency patients who have a genetic deficiency in CD18 and thus lack integrins such as LFA-1 (Hawkins *et al.*, 1992). Finally, numerous studies have demonstrated the potential of blocking antibodies directed against LFA-1 (Issekutz, 1992; Scheynius *et al.*, 1993), VLA-4 (Issekutz, 1991; Yednock *et al.*, 1992; Chisholm *et al.*, 1993), ICAM-1 (Zeng *et al.*, 1994), or VCAM (Orosz *et al.*, 1993; Silber *et al.*, 1995) to inhibit the accumulation of lymphocytes at inflammatory sites in animals.

For xenogeneic cellular interactions it is not yet fully defined which adhesion molecule interactions will function across species barriers. It is likely that at least some xenogeneic receptor ligand interactions will be nonfunctional. For example, mouse LFA-1 does not bind to human ICAM-1 (Johnston *et al.*, 1990). To explore the molecular basis of adhesive interactions between distinct classes of adhesion molecules on human and porcine cells we have cloned porcine E-selectin and VCAM-1 and have demonstrated that both are capable of mediating the adhesion of human leukocytes (Rollins *et al.*, 1994*a*; Mueller *et al.*, 1995). Further, we have developed murine monoclonal antibodies (mAbs) specific for porcine VCAM which block binding of human VLA-4 to porcine VCAM (Mueller *et al.*, 1995). The functional blocking activity of these antibodies suggests that they will be useful in suppressing the cellular response to a porcine xenograft. However, murine mAbs are not ideally suited for use in primates due to their short half-life and high immunogenicity (Khazaeli *et al.*, 1994). Here we have utilized antibody engineering strategies to create humanized anti-porcine VCAM antibodies that block adhesion of human natural killer cells, monocytes, and T lymphocytes to activated PAEC. Furthermore, we show the choice of human isotype has critical implications for the potential therapeutic use of humanized antibodies in xenotransplantation.

MATERIALS AND METHODS

Cells

Ramos, Jurkat and U-937 cells were obtained from the American Type Culture Collection, YT cells were the gift of Dr J. M. Wang (National Cancer Institute, Frederick, MD, U.S.A.), porcine aortic endothelial cells (PAEC) were obtained from Cell Systems (Kirkland, WA, U.S.A.) and 293-EBNA cells were obtained from Invitrogen (San Diego, CA, U.S.A.). U-937 cells were maintained in RPMI 1640 supplemented with 15% heat-inactivated FBS and 2 mM L-glutamine. Ramos and Jurkat cells were maintained in RPMI 1640 supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 20 mM HEPES, 50 μM β-mercaptoethanol, 100 units/ml penicillin and 100 μg/ml streptomycin. YT were maintained in RPMI 1640 containing 10% heat-inactivated FBS, 2 mM L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin. PAEC were maintained in medium A consisting of M199 supplemented with 20% heat-inactivated FBS, 0.05 mg/ml ECGF (Biomedical Technologies, Stoughton, MA, U.S.A.), 20 mM HEPES and 0.1 mg/ml heparin, and were used within three passages. 293-EBNA cells were grown in Dulbecco's modified Eagle's medium supplemented with 5% heat-inactivated FBS, 100 units/ml penicillin, 100 μg/ml streptomycin and 250 μg/ml G418. Human T cells were purified as described previously (Mueller *et al.*, 1995).

Production of VCAM/Ig fusions

A plasmid containing the human IgG1 Fc region was obtained from Dr M. J. Lenardo (NIH, Bethesda, MD, U.S.A.). DNA fragments containing various domains of human or porcine VCAM were obtained by PCR amplification of appropriate templates and joined in frame to the human IgG1 Fc coding region using standard molecular biology techniques. The cassettes encoding the fusion proteins were ligated into the expression plasmid pAPEX-3 (Evans *et al.*, 1995*a*). VCAM/Ig fusion proteins were produced by transfection of 293-EBNA cells as described previously (Evans *et al.*, 1995*a*). Three days following transfection the media were harvested, centrifuged to remove debris, and stored at 4°C. For ELISA, PolySorp 96-well microtiter plates (Nunc, Naperville, IL, U.S.A.) were coated overnight at 4°C with 100 ng/well goat anti-human IgG Fc (Sigma, St. Louis, MO, U.S.A.) in 0.1 M carbonate buffer, pH 9.6. The plates were then washed three times with PBS supplemented with 0.5% Tween 20 and incubated with blocking buffer (PBS supplemented with 0.05% Tween 20) at 37°C for 1 hr. After three further washes, various dilutions of conditioned media in blocking buffer were added and incubated at 37°C for 1 hr. Following three further washes, 2A2 or 3F4 mAb in blocking buffer was added and incubated for 1 hr at 37°C. After additional washing, peroxidase-conjugated goat anti-mouse IgG Fc (Sigma) diluted 1:2000 in blocking buffer was added and incubated at 37°C for 1 hr. After three final washes the plate was developed for 15 min with 0.4 mg/ml *o*-phenylenediamine dihydrochloride (Sigma). Reactions were stopped by addition of 1 M sulfuric acid. Quantitation was performed by measuring absorbance at 490 nm using a Bio-Rad model 3550 plate reader.

Production of chimeric antibodies

Variable regions were cloned from the 2A2 and 3F4 hybridomas by PCR amplification as described previously (Evans *et al.*, 1995*a*, 1995*b*). These sequences have been submitted to the EMBL/GenBank/DDBL under accession numbers U78799–U78802. The variable regions were joined to genomic DNA cassettes containing

Samsung Bioepis Exhibit No. 1002
Page 1157

either the intact human IgG4 constant regions or the first constant region and hinge linker region from human IgG2 followed by the second and third constant regions from human IgG4 using standard molecular biology techniques. Chimeric antibodies were produced in 293-EBNA cells and purified by chromatography on protein A Sepharose as described (Evans *et al.*, 1995*a*). Each chimeric antibody preparation was greater than 95% pure as judged by Coomassie staining of SDS polyacrylamide gels. F(ab')2 was prepared by digestion of the antibodies with ficin (Pierce, Rockford, IL, U.S.A.) followed by protein A chromatography.

Flow cytometry

Cells used were obtained by trypsinization (PAEC and 293-EBNA) or centrifugation (Ramos, Jurkat, YT and U-937 cells). Harvested cells were washed once with 4°C PBS containing 1% heat-inactivated FBS (PBS/1) and aliquoted at 50 000–100 000 cells per well in 96-well V-bottom plates (Nunc). Cells were washed two additional times with PBS/1, and then incubated with PBS/1 containing 10 μg/ml primary Ab or PBS/1 alone for 30 min at 4°C. For some experiments the chimeric HuG4 or HuG2/G4 was centrifuged at $1 \times 10^5 g$ for 1 hr prior to addition to the cells. The cells were then washed twice with PBS/1 and resuspended in PBS/1 containing the appropriate FITC-conjugated secondary antibodies (1:200 dilution) for 30 min at 4°C. The cells were again washed twice with PBS/1 and resuspended in PBS. Cell fluorescence was determined using a Becton Dickinson FACSort.

Adhesion assays

On day 0 PAEC were plated in medium A at a density of 10 000 cells per well in a 96-well flat bottom plate. On day 1 the PAEC were refed medium A supplemented with 1 μg/ml LPS (Sigma, *E. coli* 0111:B4). On day 2, human cells were resuspended in the appropriate growth medium supplemented with 10 μM calcein AM (Molecular Probes, Eugene, OR, U.S.A.) and incubated at 37°C for 30 min. The cells were subsequently washed three times with growth medium and resuspended at 6×10^6 cells/ml in RPMI 1640 supplemented with 1% heat-inactivated FBS (RPMI/1). The PAEC were washed twice with RPMI/1 and refed 50 μl/well of RPMI/1 containing the indicated concentrations of antibodies. After adding calcein-labeled cells at a density of 3×10^5 cells/well (50 μl), the plate was centrifuged at 200*g* for 1 min and incubated at 37°C for 15 min. The nonadherent cells were removed by washing three to five times with RPMI/1. Adherent cells were lysed with HBSS containing 1% SDS and calcein fluorescence was measured with a Millipore CytoFluor 2350 using an excitation wavelength of 485 nm and an emission wavelength of 530 nm. Background fluorescence was determined from wells containing PAEC that did not receive calcein-labeled cells. Samples were performed in duplicate in each experiment. Adherence was determined by comparing the residual fluorescence concentration in each well to the input fluorescence concentration. In each experiment the specific fluorescence signal obtained in the absence of competing antibody was defined as 100%.

For adhesion assays using purified soluble pVCAM (Mueller *et al.*, 1995), PolySorp 96-well microtiter plates (Nunc) were coated overnight at 4°C with 200 ng/well purified soluble pVCAM in 0.1 M carbonate buffer, pH 9.6. After blocking the plate with RPMI 1640 supplemented with 10 mg/ml BSA (RPMI/BSA), the plates were incubated with RPMI/BSA containing antibody for 1 hr at 37°C. Subsequently, 3×10^5 calcein-labeled U-937 cells in RPMI/BSA were added to each well. The plate was centrifuged upright for 1 min at 200*g* and incubated at 37°C for 15 min. The plate was then centrifuged inverted at 200*g* for 3 min. Adherent cells were lysed by the addition of HBSS containing 1% SDS and calcein fluorescence was measured with a Millipore CytoFluor 2350 using an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

RESULTS

Identification of porcine VCAM domains recognized by blocking mAbs

Previously, we have identified two murine mAbs (3F4 and 2A2) directed against porcine VCAM (pVCAM) which block VLA-4 dependent adhesion of peripheral blood human T cells to PAEC. These two mAbs are specific for pVCAM and do not recognize human VCAM (hVCAM). Here an initial analysis of the epitope specificity of the 2A2 and 3F4 mAbs was performed using fusion proteins consisting of various domains of pVCAM or hVCAM joined to the Fc region of human IgG1. The VCAM/Ig fusion proteins were produced in 293-EBNA cells and assayed for binding of 2A2 and 3F4 mAbs by ELISA (Fig. 1). Both the 2A2 and 3F4 mAbs bound efficiently to proteins containing the first three domains of pVCAM. The 2A2 mAb bound to fusion proteins containing pVCAM domain 1 such as pD1+pD2 (porcine domain 1 plus porcine domain 2) and pD1+hD2 (porcine domain 1 plus human domain 2), but did not bind to fusion proteins lacking pVCAM domain 1 such as pD2+pD3, hD1+pD2, or hD1+hD2. Weak binding of the 2A2 mAb could also be detected to a fusion protein containing only porcine domain 1 (pD1) but not to fusion proteins containing only pD2 or pD3. Conversely, the 3F4 mAb bound to fusion proteins containing pVCAM domain 2, such as pD1+pD2, pD2+pD3, and hD1+pD2, but did not bind to fusion proteins lacking pVCAM domain 2, such as pD1+hD2 and hD1+hD2. Binding of the 3F4 mAb could not be detected to fusion proteins containing only a single pVCAM domain. The weak binding of 2A2 and 3F4 to fusion proteins containing only a single VCAM domain is similar to previously characterized blocking antibodies specific for human VCAM and ICAM-1 which require the presence of at least two adhesion molecule domains for binding (Pepinsky *et al.*, 1992; Renz *et al.*, 1994). Together, these results suggested the 2A2 mAb specificity is defined by

Samsung Bioepis Exhibit No. 1002
Page 1158

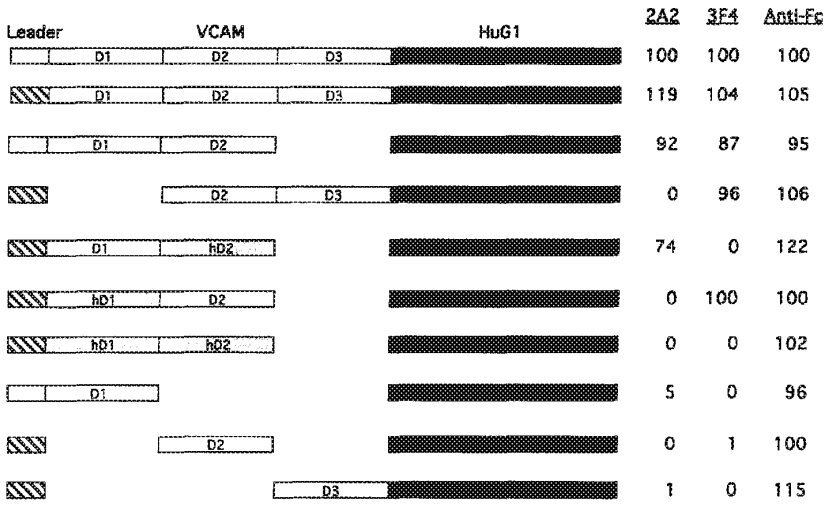


Fig. 1. Binding of 3F4 and 2A2 mAbs to VCAM/Ig fusion proteins. Proteins consisting of domains 1 (D1), 2 (D2), or 3 (D3) from porcine VCAM (open boxes) or human VCAM (gray boxes) joined to the Fc region of human IgG1 (black boxes) were produced in 293-EBNA cells. In some constructs the pVCAM secretory leader sequence was replaced with the secretory leader sequence from human CD59 (striped boxes). The fusion proteins were captured by incubating diluted conditioned media in 96-well plates coated with goat anti-human IgG (Fc specific). After incubation with 2A2 or 3F4 mAb, bound murine mAb was detected using peroxidase-conjugated goat anti-mouse IgG (Fc specific). The total amount of each fusion protein captured per well was quantitated using peroxidase-conjugated goat anti-human IgG (Fc specific). The absorbance signal obtained with the fusion protein containing the intact pVCAM domains 1, 2 and 3 was defined as 100% for each antibody. Binding of the 2A2 and 3F4 mAbs to the remaining fusion proteins is reported as a percentage of this value. Results shown are from a representative experiment of several performed.

pVCAM domain 1 whereas the 3F4 mAb specificity is defined by pVCAM domain 2.

Inhibition of human leukocyte binding to PAEC by chimeric anti-porcine VCAM mAbs

The use of murine antibodies in porcine-to-primate xenotransplantation models or ultimately in porcine-to-human xenotransplants poses several problems including a short half-life of the murine antibody plus development of an immune response directed against the murine antibody. These problems have been significantly reduced by the use of chimeric antibodies consisting of the murine variable regions joined to human constant regions (Khazaeli *et al.*, 1994). To create anti-pVCAM chimeric antibodies we cloned the variable regions from both 2A2 and 3F4. The murine variable heavy regions were joined to the human IgG4 constant region (HuG4) since this isotype does not activate complement (C) (Tao *et al.*, 1993), the predominant mediator of hyperacute rejection of xenografts. The murine variable light regions were joined to the human kappa constant region. Chimeric ch2A2 HuG4 and ch3F4 HuG4 antibodies were produced by coexpression of the chimeric heavy and light chains in 293-EBNA cells and tested for binding to pVCAM by FACS analysis using 293 cells expressing pVCAM (Fig. 2(A)). Both chimeric mAbs produced identical staining profiles as the corresponding murine mAbs, while neither chimeric mAb bound to untransfected 293 cells. Additionally, both the chimeric antibodies and murine mAbs produced identical FACS profiles using LPS-activated porcine aortic endothelial cells (Fig. 2(B)), confirming the chimeric antibodies retained the ability to bind to pVCAM expressed on PAEC.

The ability of the ch2A2 HuG4 and ch3F4 HuG4 antibodies to block the interaction between human VLA-4 and porcine VCAM was assessed by measuring inhibition of Ramos cell binding to activated PAEC (Fig. 3). The murine 3F4 mAb inhibited this binding by 90% when present at concentrations greater than 5 μg/ml. A control humanized antibody directed against human C5 (h5G1.1 HuG4) did not inhibit the binding of Ramos to activated PAEC. The ch2A2 HuG4 and ch3F4 HuG4 produced nearly identical inhibition curves as the murine 3F4 mAb, demonstrating the retention of binding avidity and cell adhesion blocking capabilities in these chimeric antibodies.

The ability of the ch2A2 HuG4 and ch3F4 HuG4 mAbs to inhibit binding of cells expressing both VLA-4 and LFA-1 was next examined using the Jurkat T cell line. Both the ch2A2 HuG4 and ch3F4 HuG4 antibodies inhibited the binding of Jurkat to activated PAEC by up to 90% while the control antibody h5G1.1 HuG4 did not inhibit binding at any concentration (Fig. 4(A)). Similarly, the ch2A2 HuG4 and ch3F4 HuG4 antibodies inhibited the binding of both resting (Fig. 4(B)) and activated (Fig. 4(C)) T cells to activated PAEC, although the

Samsung Bioepis Exhibit No. 1002
Page 1159

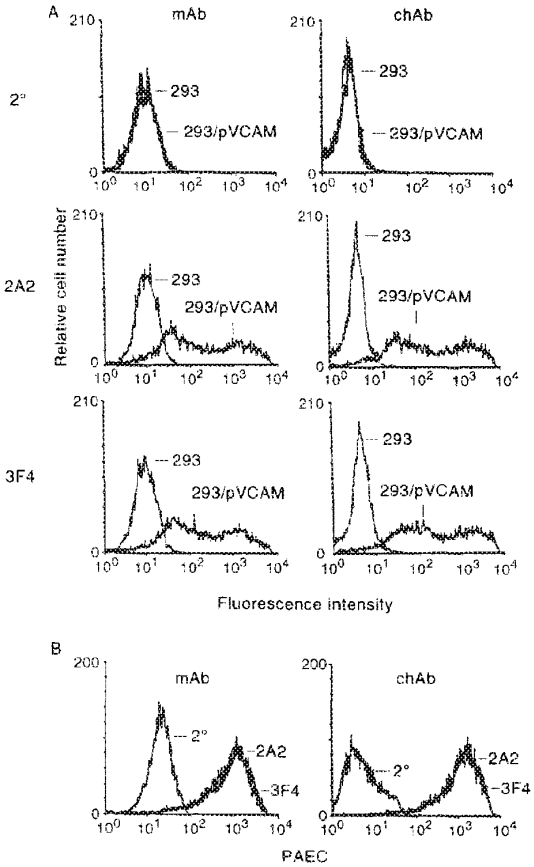


Fig. 2. Binding of 3F4 and 2A2 chimeric antibodies to pVCAM. (A) Chimeric mAbs containing the variable regions of either 2A2 or 3F4 joined to human IgG4 and kappa constant regions were produced in 293-EBNA cells and purified by protein G Sepharose chromatography. Untransfected 293-EBNA cells (293) or 293-EBNA cells expressing pVCAM (293/pVCAM) were incubated with 10 μg/ml purified murine 2A2 mAb, murine 3F4 mAb, ch2A2 HuG4, or ch3F4 HuG4. Bound antibody was detected using either FITC-conjugated goat anti-mouse IgG or FITC-conjugated goat anti-human IgG followed by flow cytometry using a Becton Dickinson FACSort. Data are displayed as histograms with fluorescence intensity plotted on the *x*-axis and relative cell number plotted on the *y*-axis. Background staining by the secondary antibodies is shown in the panels labeled 2°. (B) PAEC were activated by treatment with 1 μg/ml LPS for approximately 16 hr. Cells were then stained with either the murine 2A2 and 3F4 mAbs (left panel) or the ch2A2 HuG4 and ch3F4 HuG4 antibodies (right panel). Background staining by the secondary antibodies (2°) is indicated.

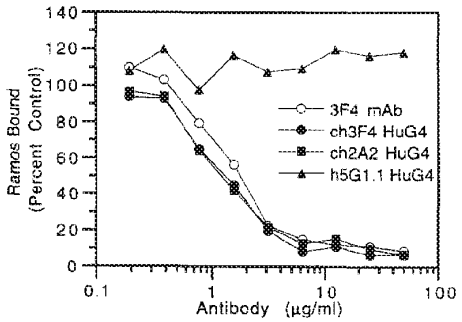


Fig. 3. Binding of Ramos cells to PAEC in the presence of mAbs. On day 0, PAEC were plated at 10000 cells per well in medium A in 96-well plates. On day 1 the PAEC were activated by adding fresh medium A supplemented with 1 μg/ml LPS. After 16 to 24 hr incubation, the PAEC were washed and refed RPMI 1640 containing 1% FCS plus the indicated concentrations of the murine 3F4 mAb (3F4 mAb), the chimeric 2A2 and 3F4 mAbs (ch2A2 HuG4 and ch3F4 HuG4) or a humanized negative control antibody (h5G1.1 HuG4). Calcein-labeled Ramos cells were added and allowed to adhere to the PAEC under static conditions for 15 min. After nonadherent cells were removed by washing, the remaining bound cells were lysed with SDS. Calcein fluorescence of the SDS supernatant was measured with a Millipore CytoFluor 2350. In each experiment the binding of Ramos cells to PAEC in the absence of antibody was defined as 100%. Results shown are the mean of two to four experiments, with each value determined in duplicate in each experiment.

maximal inhibition achieved was reduced to approximately 60%. Addition of a blocking mAb directed against human VLA-4 also maximally inhibited adhesion by 60% (Fig. 4(C)), confirming the binding inhibition observed was due to blockade of the molecular interaction between human VLA-4 and porcine VCAM.

The potential of the ch2A2 HuG4 and ch3F4 HuG4 antibodies to inhibit binding of natural killer cells and monocytes to activated PAEC was examined using the cell lines YT (Yodio *et al.*, 1985) and U-937 (Sundstron and Nilsson, 1976), respectively. Binding of YT cells to activated PAEC was strongly inhibited by concentrations of 10 μg/ml ch2A2 HuG4 and ch3F4 HuG4 antibodies (Fig. 5(A)). In contrast, the ch2A2 HuG4 and ch3F4 HuG4 antibodies did not inhibit binding of U-937 cells to activated PAEC (Fig. 5(B)). One possible explanation for this failure to inhibit U-937 cell binding could be the presence of elevated levels of VLA-4 on U-937 cells. However, FACS analysis revealed the presence of comparable levels of CD49d and activated CD29 on U-937 cells as on other cell lines such as Jurkat whose adhesion to PAEC was inhibited by the chimeric anti-pVCAM antibodies (Fig. 6). Interestingly, the adhesion of U-937 to PAEC was consistently augmented by approximately 50% in all experiments. This increase was specific for the anti-pVCAM antibodies as no increase in U-937 binding to PAEC was detected in the presence of the control h5G1.1 HuG4 antibody (Fig. 5(B)). This suggested that binding of U-937 to PAEC could be increased through FcR binding to the anti-pVCAM HuG4 antibodies on the PAEC surface. In support of this hypothesis, FACS analysis confirmed the presence of significant levels of FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) on the surface of U-937 cells. In contrast, Jurkat and YT cells had no detectable Fcγ receptors while Ramos cells showed the presence of only low levels of FcγRII (Fig. 6).

Binding of the HuG4 chimeric antibodies to U-937 could be directly demonstrated by flow cytometry (Fig.

Samsung Bioepis Exhibit No. 1002
Page 1160

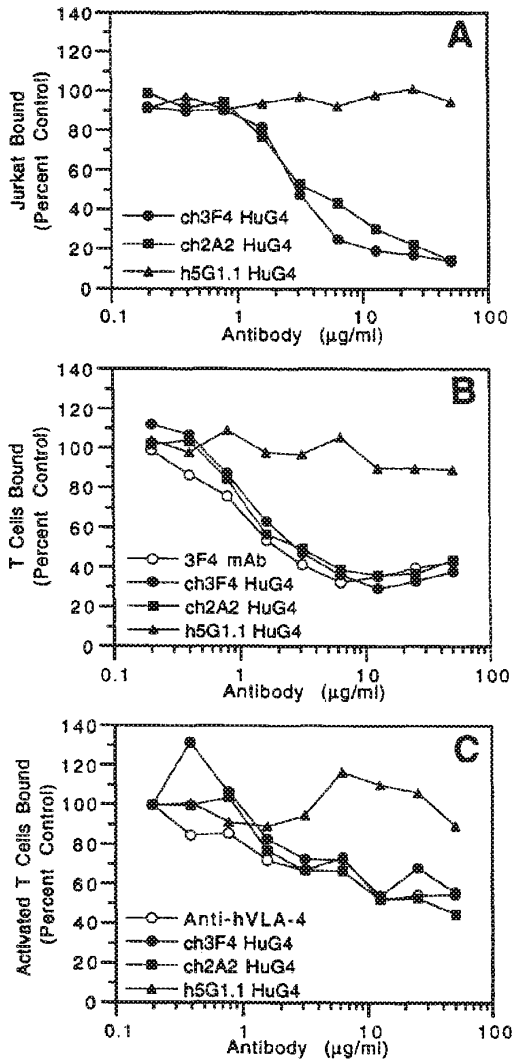


Fig. 4. Binding of Jurkat and human T cells to PAEC in the presence of HuG4 antibodies. Binding experiments were performed as described in Fig. 3 using calcein-labeled Jurkat cells (A) or calcein-labeled purified human T cells (B and C). Human T cells were either assayed immediately after purification (B) or cultured in the presence of 1 μg/ml PHA for 5 days followed by 1 day without PHA stimulation (C) prior to performing the adhesion assay. Results shown are the mean of two to four experiments (A and B) or from an individual experiment (C). Anti-hVLA-4 denotes the blocking murine mAb HP2/1 directed against the α4 subunit of human VLA-4.

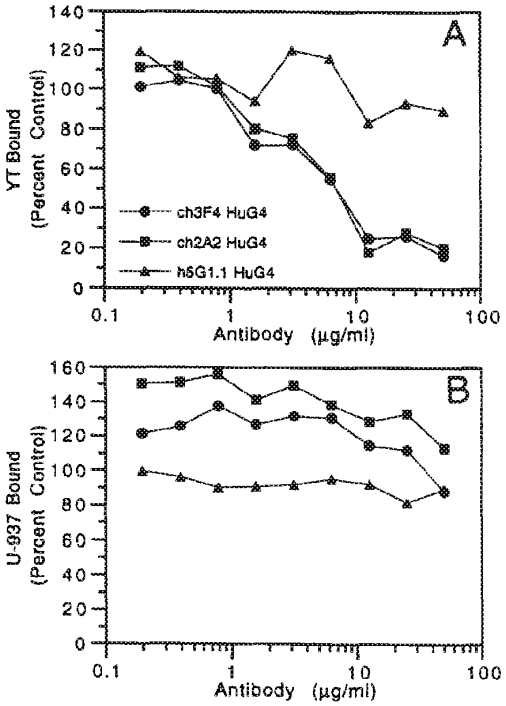


Fig. 5. Binding of YT and U-937 to PAEC in the presence of HuG4 mAbs. Adherence of calcein-labeled YT cells (A) or U-937 cells (B) to PAEC was assayed as described in Fig. 3. Results shown are the mean of two to four experiments.

7). Both the HuG4 antibodies as well as the anti-human C5 HuG4 antibody bound to U-937 cells. In contrast, 293/pVCAM cells bound only the anti-pVCAM antibodies but not the anti-human C5 HuG4 antibody. No binding of the HuG4 chimeric antibodies could be detected with Ramos, Jurkat, or YT cells. These results suggest that the HuG4 chimeric antibodies bound to U-937 cells through interaction of the HuG4 Fc region with the Fc receptors (FcRs) present on U-937 cells. Finally, F(ab′)2 fragments prepared from ch3F4 HuG4 strongly inhibited binding of U-937 cells to PAEC (Fig. 8) whereas F(ab′)2 fragments prepared from h5G1.1 HuG4 did not inhibit the binding. This result demonstrates that, if antibody crosslinking is prevented, blockade of the interaction between human VLA-4 and porcine VCAM can effectively inhibit the adhesion of human monocytes to PAEC.

Inhibition of FcR binding of humanized mAbs with altered constant regions

Although F(ab′)2 fragments proved capable of inhibiting adhesion of U-937 cells, antibody fragments are suboptimal for chronic treatments, as they have much shorter *in vivo* half-lives than intact antibodies (Covell *et al.*, 1986). The use of intact antibodies with human constant regions as anti-rejection agents is complicated by the capacity of such antibodies to fix C and/or to bind FcRs. Human antibodies of the G4 isotype are devoid of C activity (Tao *et al.*, 1993; Smith *et al.*, 1995). Human antibodies of the G2 isotype do not activate C or bind FcRs (Canfield and Morrison, 1991; Burton and Woof, 1992). Rather than altering the specific residues known to mediate C fixation and FcR binding, we explored the possibility of creating an antibody with a composite human constant region. To create chimeric mAbs which would not activate C or bind to FcRs, we utilized the overall similarity in the human G2 and G4 primary

Samsung Bioepis Exhibit No. 1002
Page 1161

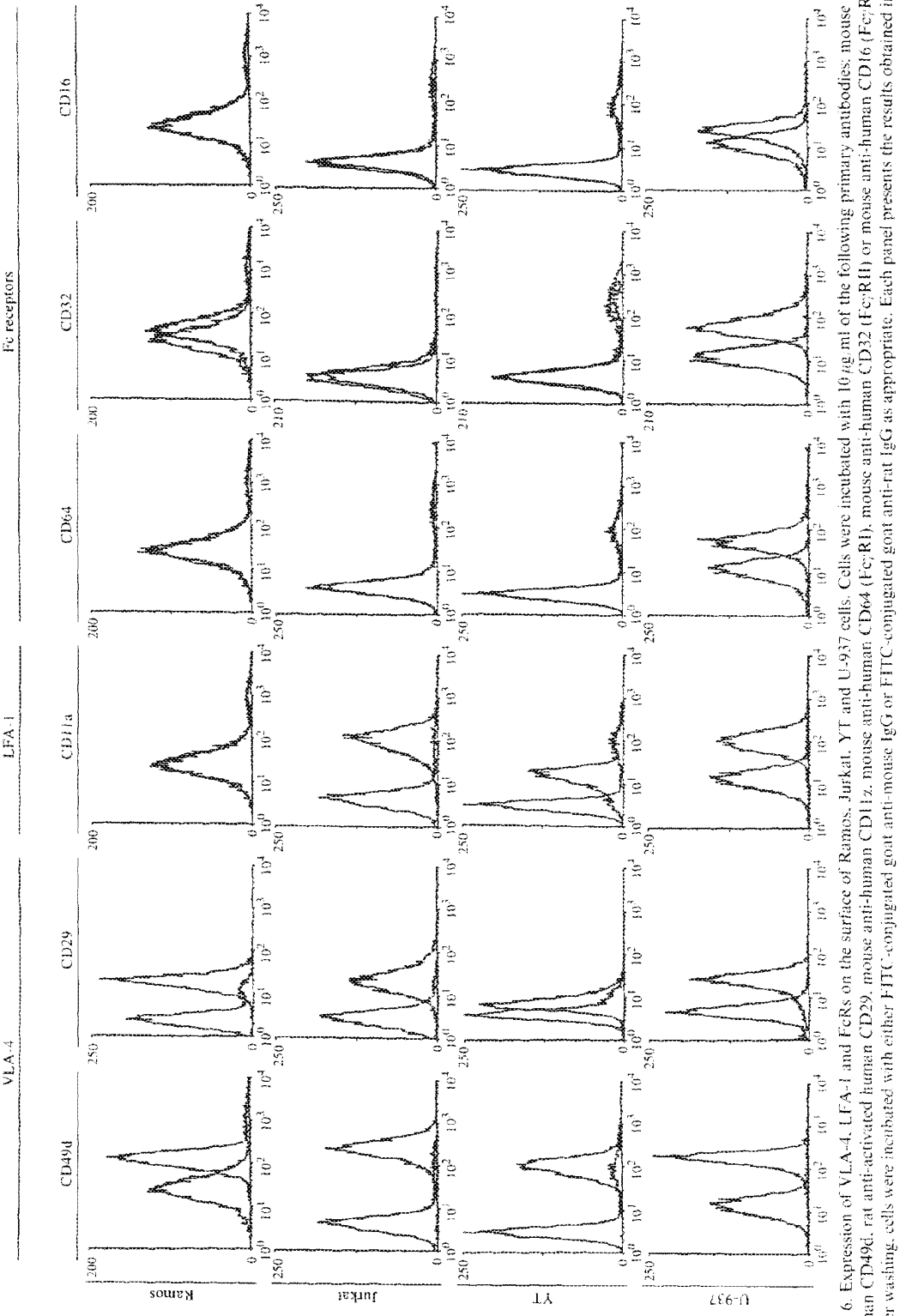


Fig. 6. Expression of VLA-4, LFA-1 and FcRs on the surface of Ramos, Jurkat, YT and U-937 cells. Cells were incubated with 10 μg ml of the following primary antibodies: mouse anti-human CD49d, rat anti-activated human CD29, mouse anti-human CD11a, mouse anti-human CD64 (FcγRI), mouse anti-human CD32 (FcγRII) or mouse anti-human CD16 (FcγRIII). After washing, cells were incubated with either FITC-conjugated goat anti-mouse IgG or FITC-conjugated goat anti-rat IgG as appropriate. Each panel presents the results obtained in the presence of primary plus secondary antibody or in the presence of secondary antibody alone.

Samsung Bioepis Exhibit No. 1002
Page 1162

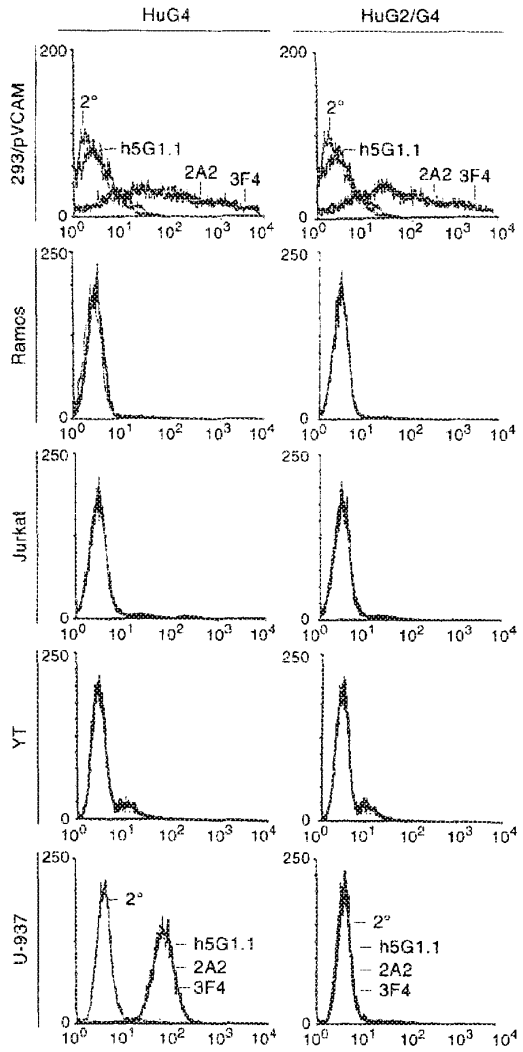


Fig. 7. Binding of chimeric antibodies to 293/pVCAM, Ramos, Jurkat, YT and U-937 cells. Cells were incubated with 10 μg/ml h5G1.1 HuG4, ch2A2 HuG4, or ch3F4 HuG4 (left panels) or 10 μg/ml h5G1.1 HuG2/G4, ch2A2 HuG2/G4, or ch3F4 HuG2/G4 (right panels). After washing, cells were incubated with FITC-conjugated goat anti-human IgG except for Ramos cells which were incubated with FITC-conjugated goat anti-human IgG (γ chain specific). Each panel presents the results obtained with the indicated cell line for h5G1.1 HuG4, ch2A2 HuG4 and ch3F4 HuG4 mAbs as well as the secondary antibody alone (2°) control.

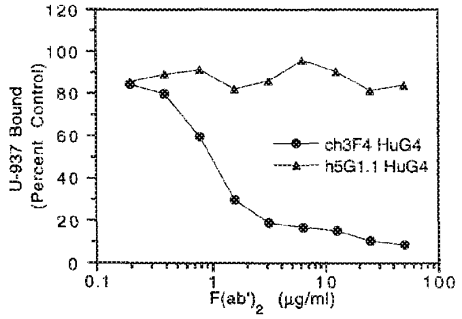


Fig. 8. Binding of U-937 cells to PAEC in the presence of F(ab')2 fragments. F(ab')2 fragments were prepared from h5G1.1 HuG4 and ch3F4 HuG4 by digestion with ficin. Undigested antibody and Fc fragments were removed by protein A chromatography. Adherence of calcein-labeled U-937 cells to PAEC in the presence of F(ab')2 was assayed as described in Fig. 3. Results shown are the mean of five experiments.

sequences to design a HuG2/G4 composite antibody consisting of the first constant region domain and the hinge region from the HuG2 isotype joined to the second and third constant region domains from the HuG4 isotype (Fig. 9). The resulting mAb did not contain the antibody sequences necessary for FcR binding, nor any new epitopes that would likely be immunogenic.

Binding to FcR was examined by using the monocytic cell line U937. FACS analysis demonstrated that the resulting ch3F4 HuG2/G4, ch2A2 HuG2/G4 and h5G1.1 HuG2/G4 antibodies did not bind to U-937 cells (Fig. 7). The ch3F4 HuG2/G4 and ch2A2 HuG2/G4 antibodies maintained the ability to bind pVCAM as demonstrated both by binding to 293/pVCAM cells (Fig. 7) and by inhibition of Jurkat and YT binding to activated PAEC (Fig. 10). For both Jurkat and YT cells inhibition of binding with the chimeric HuG2/G4 was equivalent to the HuG4 or original murine anti-pVCAM antibodies. The binding of U-937 cells to PAEC was now potently inhibited by the presence of either ch2A2 HuG2/G4 or ch3F4 HuG2/G4 antibodies. Finally, the chimeric antibodies were examined for the ability to block the adhesion of U-937 cells to purified soluble pVCAM (Fig. 11). Addition of the ch3F4 HuG4 antibody failed to inhibit binding of U-937 cells to pVCAM unless very high concentrations of antibody were added. In contrast the ch3F4 HuG2/G4 antibody potently inhibited the binding of U-937 cells to pVCAM, while the negative control h5G1.1 HuG2/G4 antibody did not block binding. Together, these results demonstrate the anti-pVCAM HuG2/G4 antibodies specifically inhibited the adhesive interaction of human VLA-4 with porcine VCAM and that disruption of this interaction was sufficient to inhibit binding of numerous human cell types to porcine endothelial cells.

DISCUSSION

Current success in allotransplantation has resulted from the development of effective global immunosuppressive therapies that prevent cellular graft rejection. Recent attempts to develop more specific immunosuppressive strategies have focused on inhibiting interactions between integrins and costimulatory molecules on T cells with their ligands on graft endothelial cells and other antigen presenting cells (reviewed in Faustman, 1995; Guinan *et al.*, 1994). The success of allotransplantation has been limited by the resulting shortage of human donor organs. Recent progress in overcoming the initial

Samsung Bioepis Exhibit No. 1002
Page 1163

```
HuG2      STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV
HuG2/G4   STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV
HuG4      STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV

HuG2      HTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVE
HuG2/G4   HTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVE
HuG4      HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVE

                          235
HuG2      RKCCVECPPCPAPPV- AGPSVFLF
HuG2/G4   RKCCVECPPCPAPPV- AGPSVFLF
HuG4      SKYGPPCPSCPAPEFLGGPSVFLF

HuG2      PPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPR
HuG2/G4   PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR
HuG4      PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR

                                                      331
HuG2      EEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKG
HuG2/G4   EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKG
HuG4      EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKG
```

Fig. 9. Sequence of the CH1, hinge and CH2 regions of human IgG2 (HuG2), human IgG4 (HuG4), and the chimeric HuG2/G4 construct. Positions where differences occur between the three molecules are double underlined. The amino acid sequence at position 235/236 determines ability to bind to Fcγ receptors (Canfield and Morrison, 1991; Duncan *et al.*, 1988; Alegre *et al.*, 1992; Burton and Woof, 1992) while the presence of a serine at position 331 confers the inability to activate C (Tao *et al.*, 1993).

hyperacute rejection of discordant xenografts suggests the potential for use of porcine xenografts as a clinical option to circumvent this chronic shortage. Although there are limited data regarding the fate of discordant xenografts which survive the initial hyperacute rejection phase, xenograft recipients will likely require continued cellular immunosuppression for the prevention of graft rejection. In this light, xenografts offer a unique opportunity for the development of blocking reagents specific for the graft, thereby avoiding global immunosuppression of the recipient.

To investigate the interactions occurring between human leukocytes and porcine endothelial cells, we have cloned the porcine VCAM molecule and created mAbs specific for porcine VCAM that do not bind to human VCAM (Mueller *et al.*, 1995). Two of these antibodies (3F4 and 2A2) are capable of blocking the interaction between human VLA-4 and porcine VCAM. Here we demonstrate that the binding specificity for 2A2 is located within domain 1 of pVCAM while the binding specificity for 3F4 is located within domain 2. This is identical to the results found with human VCAM, where blocking antibodies have also been shown to be directed against either domains 1 or 2 (Osborn *et al.*, 1992; Vonderheide and Springer, 1992).

The use of murine mAbs in humans and primates presents numerous problems including a short antibody half-life and a high degree of immunogenicity (Khazaeli *et al.*, 1994). To optimize the use of the anti-porcine VCAM antibodies in porcine-to-primate xenograft experiments we developed chimeric antibodies using the variable regions cloned from the 2A2 and 3F4 antibodies joined to the constant regions of the human IgG4,κ isotype. Given the extensive activation of C which occurs within xenografts, the human IgG4 isotype was chosen due to its inability to activate C. The resultant chimeric antibodies (ch2A2 HuG4 and ch3F4 HuG4 antibodies) retained specificity for porcine VCAM and were as potent as the original murine mAbs at blocking adhesion of Ramos cells to activated PAEC by greater than 90% (Fig. 3). In contrast to Ramos, the binding of resting or activated human T cells to activated PAEC was inhibited only approximately 50% by either the chimeric antibodies or the original mAbs (Fig. 4). Activation of human T cells by PAEC can be blocked by antibodies directed against either CD2 or LFA-1 (Rollins *et al.*, 1994*b*), suggesting either of these molecules could mediate the remaining adhesion by interacting with porcine CD58 or porcine ICAM-1, respectively.

The adhesion of human lymphocytes, monocytes or NK cells to activated HUVEC is dependent on the interaction of both LFA-1 with ICAM-1 and VLA-4 with VCAM (Allavena *et al.*, 1991; Jonjic *et al.*, 1992; Vonderheide and Springer, 1992). For each cell type, blocking the VLA-4/VCAM interaction by itself produces only a small reduction in adhesion, while antibodies which disrupt the LFA-1/ICAM-1 interaction provide a greater, although still partial, inhibition of adhesion. Addition of antibodies blocking both VLA-4/VCAM and LFA-1/ICAM-1 is required to completely block adhesion of these cell types to HUVEC. Surprisingly, binding of LFA-1$^+$–VLA$^+$4 Jurkat or YT cells to activated PAEC could be inhibited nearly completely by the ch3F4 HuG4 and ch2A2 HuG4 antibodies. One possible explanation for this could be the lack of interaction between human LFA-1 and porcine ICAM. The conservation of ICAM-1 is relatively low between species, with human and mouse ICAM-1 being only 50% conserved at the amino acid level (reviewed in Carlos and Harlan, 1994), and *in vitro* mouse LFA-1 does not bind to human ICAM-1. Alternatively, human LFA-1 may interact with porcine ICAM-1, but with a reduced affinity. For example, human VLA-4 binds to human VCAM at two independent sites, one within domain 1 and one within

Samsung Bioepis Exhibit No. 1002
Page 1164

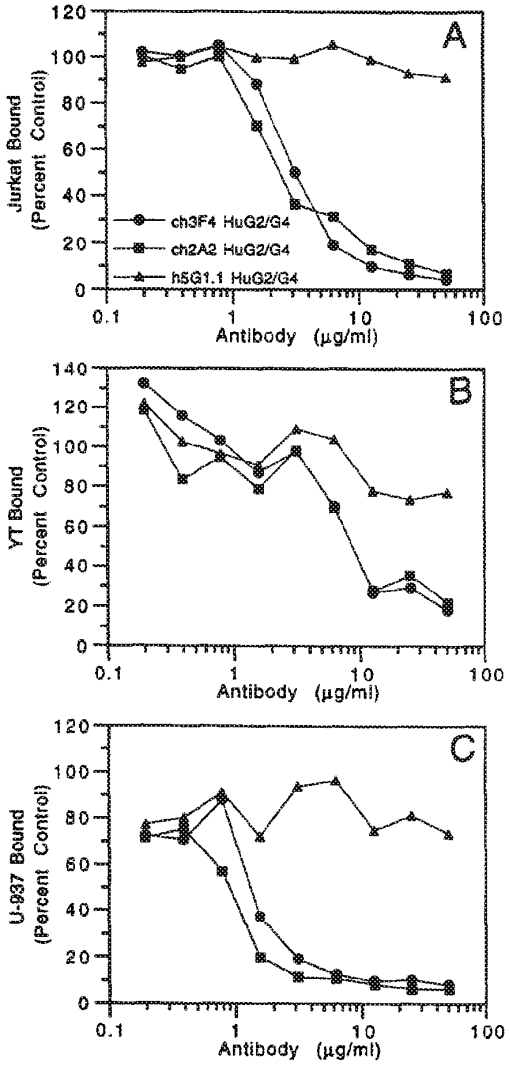


Fig. 10. Binding of Jurkat, YT and U-937 to PAEC in the presence of HuG2/G4 mAbs. Adherence of calcein-labeled Jurkat (A), YT (B) and U-937 (C) cells to PAEC was assayed as described in Fig. 3. Results shown are the mean of two to four experiments.

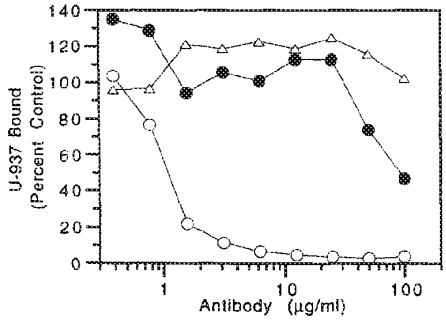


Fig. 11. Binding of U-937 to purified pVCAM. Microtiter plate wells were coated with purified soluble pVCAM. After blocking with FCS, wells received the indicated concentrations of either ch3F4 HuG4 (●), ch3F4 HuG2/G4 (○) or h5G1.1 HuG2/G4 (△) followed by 3×10^5 calcein-labeled U-937 cells. The cells were allowed to adhere at 37°C for 15 min after which non-adherent cells were removed by washing. Adherence of U-937 cells in the absence of competitor antibody was defined as 100%. Results shown are from an individual representative experiment.

domain 4 (Osborn *et al.*, 1992; Vonderheide *et al.*, 1994). In contrast, porcine VCAM lacks domain 4, suggesting the interaction between human VLA-4 and porcine VCAM may be weaker than the interaction between human VLA-4 and human VCAM. Reduced ligand affinities could make the binding of human effector cells to porcine endothelial cells more susceptible to disruption of any one of multiple weak interactions. These observations suggest the potential for significant differences in the therapeutic efficacy achieved by inhibiting particular receptor–ligand interactions with xenografts as compared to allografts.

Although the ch3F4 HuG4 and ch2A2HuG4 blocked binding of Jurkat and YT to activated PAEC, both antibodies failed to block binding of the monocytic U-937 cells to activated PAEC (Fig. 5). The levels of activated $\beta 1$ were comparable on Jurkat and U937 cells indicating an increased level of functional VLA-4 was not the mechanism behind the lack of inhibition of U-937 binding. Additionally, flow cytometry demonstrated the direct binding of the chimeric HuG4 antibodies to U-937 cells (Fig. 7). This binding was not dependent on the antigen specificity of the variable region, suggesting the interaction occurred through the Fc portion of the antibody. Finally, F(ab')2 prepared from 3F4 HuG4 were able to block the adhesion of U-937 cells to activated PAEC by greater than 80% (Fig. 8). Together these results were consistent with a model in which the chimeric anti-pVCAM HuG4 mAbs crosslinked U-937 cells to activated PAEC by simultaneously binding FcRs and cell surface VCAM present on the PAEC. This is comparable to the situation encountered with the anti-CD3 murine monoclonal antibody (mAb) OKT3, which when first administered to patients produces crosslinking of T cells to FcR bearing cells, resulting in the activation of both cell types (Ceuppens *et al.*, 1985; Kan *et al.*, 1986) with resultant toxicity to the patient.

The interaction of antibodies with Fcγ receptors has been found to be dependent on the sequence of the hinge linker region of the CH2 domain (Duncan *et al.*, 1988; Alegre *et al.*, 1992; Burton and Woof, 1992) as well as an additional bend located between two β strands within the CH2 domain (Canfield and Morrison, 1991). In the case of a humanized OKT3 antibody (human IgG4 constant regions), binding to Fcγ receptors has been eliminated by the introduction of amino acid mutations in the hinge linker (Alegre *et al.*, 1992, 1994). In either case the resulting human IgG4 constant region contains amino acid differences which could be immunogenic. Here, we have created chimeric antibodies (designated HuG2/G4) containing the CH1 domain and hinge linker region of

Samsung Bioepis Exhibit No. 1002
Page 1165

human IgG2 followed by the CH2 and CH3 domains of the human IgG4 constant region. Due to the similarities between these two isotypes at the amino acid level, the resultant antibody contained no novel peptides less than 30 amino acids in length likely to be presented by MHC class II molecules and invoke an immune response. The chimeric HuG2/G4 antibodies no longer bound to U-937 cells. Moreover, the ch2A2 HuG2/G4 and ch3F4 HuG2/G4 antibodies blocked the binding of Jurkat and YT cells to activated PAEC as potently as the chimeric HuG4 antibodies, demonstrating the alteration of the Fc region did not reduce the antibody avidities. Moreover, the chimeric anti-pVCAM HuG2/G4 recombinant antibodies nearly completely blocked the binding of U-937 cells to activated PAEC and to purified pVCAM. Finally, as predicted from the low affinity of IgG2 and IgG4 for human C1q, our preliminary results indicate that the HuG4 and HuG2/G4 recombinant antibodies show little or no C1q binding (data not shown).

In summary, we have developed humanized mAbs specific for porcine VCAM which potently block the adhesion of multiple human inflammatory cell types to porcine endothelial cells. The lack of binding of these antibodies to human VCAM leaves intact the interaction of human leukocytes with human endothelial cells. The adhesion-blocking properties of these antibodies will allow delineation of the role of VLA-4/VCAM interactions in cellular mediated rejection of porcine-to-primate xenografts. Additionally, the HuG2/G4 antibody design should prove useful in humanization of other antibodies intended for human use where elimination of FcR binding and C activation may be desirable.

REFERENCES

Alegre M.-L., Collins A. M., Pulito V. L., Brosius R. A., Olson W. C., Zivin R. A., Knowles R., Thistlethwaite J. R., Jolliffe L. K. and Bluestone J. A. (1992) Effect of a single amino acid mutation on the activating and immunosuppressive properties of a "humanized" OKT3 mAb. *J. Immun.* **148,** 3461–3468.

Alegre M.-L., Peterson L. J., Xu D., Sattar H. A., Jeharajah D. R., Kowalkowski K., Thistlethwaite J. R., Zivin R. A., Jolliffe L. and Bluestone J. A. (1994) A non-activating "humanized" anti-CD3 mAb retains immunosuppressive properties *in vivo*. *Transplantation* **57,** 1537–1543.

Allavena P., Paganin C., Martin-Padura I., Peri G., Baboli M., Dejana E., Marchisio P. C. and Montovani A. (1991) Molecules and structures involved in the adhesion of natural killer cells to vascular endothelium. *J. Exp. Med.* **173,** 439–448.

Alon R., Kassner P. D., Carr M. W., Finger E. B., Hemlet M. E. and Springer T. A. (1995) The integrin VLA-4 supports tethering and rolling in flow on VCAM-1. *J. Cell Biol.* **128,** 1243–1253.

Bianchi G., Sironi M., Ghibaudi E., Selvaggini C., Elices M., Allavena P. and Montovani A. (1993) Migration of natural killer cells across endothelial cell monolayers. *J. Immun.* **151,** 5135–5144.

Blakely M. L., Werf W. J. van der, Berndt M. C., Dalmasso A. P., Bach F. H. and Hancock W. W. (1994) Activation of intragraft endothelial and mononuclear cells during discordant xenograft rejection. *Transplantation* **58,** 1059–1066.

Burton D. R. and Woof J. M. (1992) Human antibody effector function. *Adv. Immun.* **51,** 1–18.

Canfield S. M. and Morrison S. L. (1991) The binding affinity of human IgG for its high affinity FcR is determined by multiple amino acids in the CH2 domain and is modulated by the hinge region. *J. Exp. Med.* **173,** 1483–1491.

Carlos T. M. and Harlan J. M. (1994) Leukocyte-endothelial adhesion molecules. *Blood* **84,** 2068–2101.

Ceuppens J. L., Bloemmen F. J. and Wauwe J. P. van (1985) T cell unresponsiveness to the mitogenic activity of OKT3 antibody results from a deficiency of monocyte Fcγ receptors for murine IgG2a and inability to cross-link the T3–Ti complex. *J. Immun.* **135,** 3882–3886.

Chisholm P. L., Williams C. A. and Lobb R. R. (1993) Monoclonal antibodies to the integrin α4 subunit inhibit the murine contact hypersensitivity response. *Eur. J. Immun.* **23,** 621–628.

Chuluyan H. E., Osborn L., Lobb R. and Issekutz A. C. (1995) Domains 1 and 4 of vascular cell adhesion molecule-1 (CD106) both support very late activation antigen-4 (CD49d/CD29)-dependent monocyte transendothelial migration. *J. Immun.* **155,** 3135–3144

Covell D. G., Barbet J., Holton O. D., Black C. D., Parker P. J. and Weinstein J. N. (1986) Pharmacokinetics of monoclonal immunoglobulin G1, F(ab')2, and Fab' in mice. *Cancer Res.* **46,** 3969–3978.

Duncan A. R., Woof J. M., Partridge L. J., Burton D. R. and Winter G. (1988) Localization of the binding site for the human high-affinity FcR on IgG. *Nature* **332,** 563–564.

Evans M. J., Hartman S. L., Wolff D. W., Rollins S. A. and Squinto S. P. (1995*a*) Rapid expression of an anti-human C5 chimeric Fab utilizing a vector that replicates in COS and 293 cells. *J. Immun. Meth.* **184,** 123–138.

Evans M. J., Rollins S. A., Wolff D. W., Rother R. P., Norin A. J., Thernen D. M., Grijalva G. A., Mueller J. P., Nye S. H., Squinto S. P. and Wilkins J. A. (1995*b*) *In vitro* and *in vivo* inhibition of C activity by a single-chain Fv fragment recognizing human C5. *Mol. Immun.* **32,** 1183–1195.

Faustman D. (1995) Strategies for circumventing transplant rejection: modification of cells, tissues and organs. *Trends Biotech.* **13,** 100–105.

Guinan E. C., Gribben J. G., Boussiotis V. A., Freeman G. J. and Nadler L. M. (1994) Pivotal role of the B7:CD28 pathway in transplantation tolerance and tumor immunity. *Blood* **84,** 3261–3282.

Hawkins H. K., Heffelfinger S. C. and Anderson D. C. (1992) Leukocyte adhesion deficiency: clinical and postmortem observations. *Ped. Pathol.* **12,** 119–130.

Issekutz T. B. (1991) Inhibition of *in vivo* lymphocyte migration to inflammation and homing to lymphoid tissues by the TA-2 mAb: a likely role for VLA-4 *in vivo*. *J. Immun.* **147,** 4178–4184.

Issekutz T. B. (1992) Inhibition of lymphocyte endothelial adhesion and *in vivo* lymphocyte migration to cutaneous inflammation by TA-3, a new mAb to rat LFA-1. *J. Immun.* **149,** 3394–3402.

Johnston S. C., Dustin M. L., Hibbs M. L. and Springer T. A. (1990) On the species specificity of the interaction of LFA-1 with intercellular adhesion molecules. *J. Immun.* **145,** 1181–1187.

Jonjic N., Jilec P., Bernasconi S., Peri G., Martin-Padura I., Cenzuales S., Dejana E. and Mantovani A. (1992) Molecules involved in the adhesion and cytotoxicity of activated monocytes on endothelial cells. *J. Immun.* **148,** 2080–2083.

Samsung Bioepis Exhibit No. 1002
Page 1166

Kan E. A. R., Wright S. D., Welte K. and Wand C. Y. (1986) FcRs on monocytes cause OKT3-treated lymphocytes to internalize T3 and to secrete IL-2. *Cell. Immun.* **98,** 181–185.

Khazaeli M. B., Conry R. M. and LoBuglio A. F. (1994) Human immune response to mAbs. *J. Immunother.* **15,** 42–52.

Luscinskas F. W., Kansas G. S., Ding H., Pizcueta P., Schleiffenbaum B. E., Tedder T. F. and Gimbrone M. A. (1994) Monocyte rolling, arrest and spreading on IL-4-activated vascular endothelium under flow is mediated via sequential action of L-selectin, β1-integrins, and β2-integrins. *J. Cell Biol.* **125,** 1417–1427.

Luscinskas F. W., Ding H. and Lichtman A. H. (1995) P-selectin and vascular cell adhesion molecule 1 mediate rolling and arrest, respectively, of CD4+ T lymphocytes on tumor necrosis factor α-activated vascular endothelium under flow. *J. Exp. Med.* **181,** 1179–1186.

Meershaert J. and Furie M. B. (1994) Monocytes use either CD11/CD18 or VLA-4 to migrate across human endothelium *in vitro*. *J. Immun.* **152,** 1915–1926.

Mueller J. P., Evans M. J., Cofiell R., Rother R. P., Matis L. A. and Elliott E. A. (1995) Porcine vascular cell adhesion molecule (VCAM) mediates endothelial cell adhesion to human T cells. *Transplantation* **60,** 1299–1306.

Murray A. G., Khodadoust M. M., Pober J. S. and Bothwell A. L. (1994) Porcine aortic endothelial cells activate human T cells: direct presentation of MHC antigens and costimulation by ligands for human CD2 and CD28. *Immunity* **1,** 57–63.

Orosz C. G., Ohye R. G., Pelletier R. P., van Buskirk A. M., Huang E., Morgan C., Kincade P. W. and Ferguson R. M. (1993) Treatment with anti-vascular cell adhesion molecule 1 mAb induces long-term murine cardiac allograft acceptance. *Transplantation* **56,** 453–460.

Osborn L., Vassallo C. and Benjamin C. D. (1992) Activated endothelium binds lymphocytes through a novel binding site in the alternately spliced domain of vascular cell adhesion molecule-1. *J. Exp. Med.* **176,** 99–107.

Pepinsky B., Hession C., Chen L.-L., Moy P., Burkly L., Jakubowski A., Chow E. P., Benjamin C., Chi-Rosso G., Luhowskyj S. and Lobb R. (1992) Structure/function studies on vascular cell adhesion molecule-1. *J. Biol. Chem.* **267,** 17820–17826.

Renz M. E., Chiu H. H., Jones S., Fox J., Kin K. J., Presta L. G. and Fong S. (1994) Structural requirements for adhesion of soluble recombinant murine vascular cell adhesion molecule-1 to $\alpha 4\beta 1$. *J. Cell Biol.* **125,** 1395–1406.

Rollins S. A., Evans M. J., Johnson K. K., Elliott E. A., Squinto S. P., Matis L. A. and Rother R. P. (1994*a*) Molecular and functional analysis of porcine E-selectin reveals a potential role in xenograft rejection. *Biochem. Biophys. Res. Commun.* **204,** 763–771.

Rollins S. A., Kennedy S. P., Chodera A. J., Elliott E. A., Zavoico G. B. and Matis L. A. (1994*b*) Evidence that activation of human T cells by porcine endothelium involves direct recognition of porcine SLA and costimulation by porcine ligands for LFA-1 and CD2. *Transplantation* **57,** 1709–1716.

Scheynius A., Camp R. L. and Puré E. (1993) Reduced contact sensitivity reactions in mice treated with mAbs to leukocyte function-associated molecule-1 and intercellular adhesion molecule-1. *J. Immun.* **150,** 655–663.

Silber A., Newman W., Sasseville V. G., Pauley D., Beall D., Walsh D. G. and Ringler D. J. (1995) Recruitment of lymphocytes during cutaneous delayed hypersensitivity in non-human primates is dependent on E-selectin and VCAM-1. *J. Clin. Invest.* **93,** 1554–1563.

Smith R. I. F., Coloma M. J. and Morrison S. L. (1995) Addition of a μ-tailpiece to IgG results in polymeric antibodies with enhanced effector functions including C-mediated cytolysis by IgG4. *J. Immun.* **154,** 2226–2236.

Somerville C. A. and D'Apice A. J. F. (1993) Future directions in transplantation: xenotransplantation. *Kidney Int.* **42,** S112.

Sundstron C. and Nilsson K. (1976) Establishment and characterization of a human histiocytic lymphoma cell line (U-937). *Int. J. Cancer* **17,** 565–569.

Tao M.-H., Smith R. I. F. and Morrison S. L. (1993) Structural features of human immunoglobulin G that determine isotype-specific differences in C activation. *J. Exp. Med.* **178,** 661–667.

Vonderheide R. H., Springer T. A. (1992) Lymphocyte adhesion through very late antigen 4: evidence for a novel binding site in the alternatively spliced domain of vascular cell adhesion molecule 1 and an additional a4 integrin counter-receptor on stimulated endothelium. *J. Exp. Med.* **175,** 1422–1433.

Vonderheide R. H., Tedder T. F., Springer T. A. and Staunton D. E. (1994) Residues within a conserved amino acid motif of domains 1 and 4 of VCAM-1 are required for binding to VLA-4. *J. Cell Biol.* **125,** 215–222.

Waligren A. C., Karlssonparra A. and Korsgren O. (1995) The main infiltrating cell in xenograft rejection is a CD4(+) macrophage and not a T lymphocyte. *Transplantation* **60,** 594–601.

Yamada K., Sachs D. H. and DerSimonian H. (1995) Human anti-porcine xenogeneic T cell response. Evidence for allele specificity of mixed leukocyte reaction and for both direct and indirect pathways of recognition. *J. Immun.* **155,** 5249–5256.

Yednock T. A., Cannon C., Fritz L. C., Sanchex-Madrid F., Steinman L. and Karin N. (1992) Prevention of experimental autoimmune encephalomyelitis by antibodies against $\alpha 4\beta 1$ integrin. *Nature* **356,** 63–66.

Yodio J., Teshigawara K., Nikaido T., Fukui J. K., Noma T., Honjo T., Takigawa M., Minato M., Tsudo M., Uchiyama T. and Maeda M. (1985) TCGF (IL-2)-receptor inducing factor(s) I. Regulation of IL-2 receptor on a natural killer-like cell line (YT cells). *J. Immun.* **164,** 1623–1627.

Zeng Y., Gage A., Montag A., Rothlein R., Thistlethwaite J. R. and Bluestone J. A. (1994) Inhibition of transplant rejection by pretreatment of xenogeneic pancreatic islet cells with anti-ICAM-1 antibodies. *Transplantation* **58,** 681–689.

Samsung Bioepis Exhibit No. 1002
Page 1167

nature biotechnology

Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria

Russell P Rother[1], Scott A Rollins[1], Christopher F Mojcik[1], Robert A Brodsky[2] & Leonard Bell[1]

The complement system provides critical immunoprotective and immunoregulatory functions but uncontrolled complement activation can lead to severe pathology. In the rare hemolytic disease paroxysmal nocturnal hemoglobinuria (PNH), somatic mutations result in a deficiency of glycosylphosphatidylinositol-linked surface proteins, including the terminal complement inhibitor CD59, on hematopoietic stem cells. In a dysfunctional bone marrow background, these mutated progenitor blood cells expand and populate the periphery. Deficiency of CD59 on PNH red blood cells results in chronic complement-mediated intravascular hemolysis, a process central to the morbidity and mortality of PNH. A recently developed, humanized monoclonal antibody directed against complement component C5, eculizumab (Soliris; Alexion Pharmaceuticals Inc., Cheshire, CT, USA), blocks the proinflammatory and cytolytic effects of terminal complement activation. The recent approval of eculizumab as a first-in-class complement inhibitor for the treatment of PNH validates the concept of complement inhibition as an effective therapy and provides rationale for investigation of other indications in which complement plays a role.

The complement system: a potential target for therapeutics

The complement system consists of more than 20 serum proteins that interact in a precise series of enzymatic cleavage and membrane binding events leading to the generation of products with immunoprotective, immunoregulatory, proinflammatory and cytolytic properties[1,2].

Proximal complement. The cascade proceeds through three distinct pathways—alternative, classical and lectin—which all result in the generation of C3 convertase complexes that mediate the cleavage of C3 to C3a and C3b[1,3] (**Fig. 1**). Recent studies have delineated the structure of C3 and C3 fragments and provided important insights into their function[4–7]. The classical pathway is generally initiated by the interaction of C1q with antigen-antibody complexes. The lectin pathway is activated by the interaction of mannose-binding lectin (MBL) with specific mannose-containing carbohydrate structures. The alternative pathway is initiated by deposition of preformed C3b on a variety of substrates such as bacteria and cell membranes, including erythrocytes. This C3b is continuously available due to the interaction of C3 with water, a process that represents an important part of the innate immune system called complement 'tick-over'. In addition to its role in the initiation of alternative pathway activation, C3b is also necessary for the amplification and progression of the complement cascade through all pathways of activation and serves as a key immunoprotective and immunoregulatory molecule.

The importance of proximal complement components in immunity is illustrated by the clinical effects observed in people with genetic complement deficiencies. For example, individuals with C3 deficiency are at particular risk of infection from polysaccharide-coated bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis*, and frequently die at a young age from overwhelming sepsis[8]. The absence of C3 is generally thought to prevent the progression of complement through all pathways of activation, thereby reducing the body's ability to opsonize pathogens and mount an inflammatory response, although some studies suggest that C5 cleavage can occur in the absence of C3 by thrombin[3,9]. Individuals deficient in C1, C2 or C4 develop severe autoimmune symptoms such as a lupus-like syndrome and are also susceptible to recurrent overwhelming sepsis. Thus, deficiencies in early components of the complement cascade lead to impairment in the ability to generate C3b, which is critical for immune complex clearance and is the primary opsonin for many pathogenic microorganisms.

Terminal complement. All pathways of complement activation converge at the cleavage of C5 into C5a and C5b by the C5 convertase enzyme complexes. This cleavage event initiates the terminal complement cascade (**Fig. 1**). C5a is a potent anaphylatoxin that mediates leukocyte chemotaxis, increases vascular permeability, alters smooth muscle tone and induces secondary inflammatory mediators such as hydrolytic enzymes, reactive oxygen species, arachidonic acid metabolites and cytokines[10]. C5a receptors are present on a variety of cell types that directly contribute to inflammation, including monocytes, macrophages and neutrophils. The C5a receptor CD88 is a member of the N-formyl peptide receptor family, which transduces signals through a G protein–dependent pathway resulting in biochemical proinflammatory

[1]Alexion Pharmaceuticals, Inc., 352 Knotter Drive, Cheshire, Connecticut 06410, USA. [2]Johns Hopkins University School of Medicine, Division of Hematology, 720 Rutland Avenue, Ross Building Room 1025, Baltimore, Maryland 21205, USA. Correspondence should be addressed to R.P.R. (rotherr@alxn.com).

Published online 7 November; corrected after print 12 December 2007; doi:10.1038/nbt1344

Samsung Bioepis Exhibit No. 1002
Page 1168

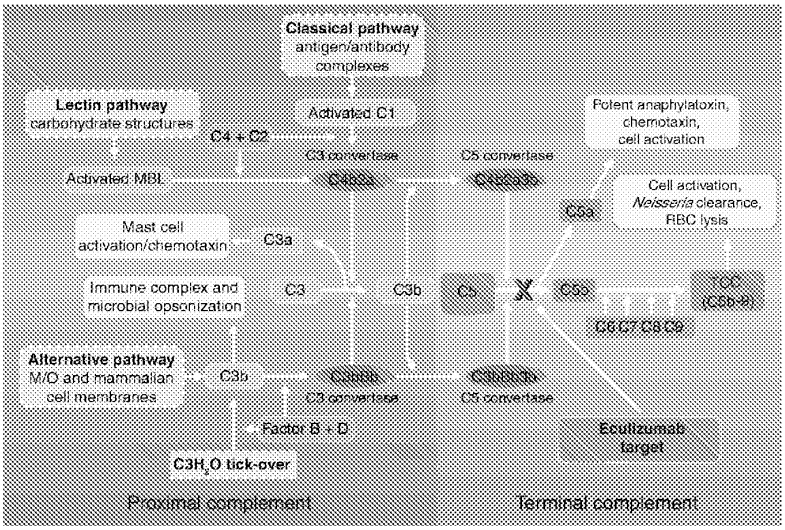


Figure 1 Targeted blockade of complement protein C5. The complement cascade can be activated via the classical, lectin and alternative pathways. The proximal components of complement (proteins upstream of C5) are essential for microbial opsonization and immune complex clearance. All pathways of complement activation converge at the cleavage of the terminal complement protein C5 leading to the generation of molecules with proinflammatory and cell lytic properties. Targeted blockade at C5 with eculizumab therefore prevents the deleterious properties of terminal complement activation while preserving the immunoprotective and immunoregulatory functions of proximal complement. M/O, microorganisms. TCC, terminal complement complex.

responses[11]. A second C5a receptor has been identified (C5L2) that also facilitates C5a-mediated signaling in a variety of cell types[12]. C5b recruits the terminal complement components C6, C7, C8 and C9 to form C5b-9 or the terminal complement complex (TCC) on the surface of cells. The generation of the TCC at sublytic concentrations stimulates the release of many of the same proinflammatory molecules described for C5a. Most importantly, unimpeded assembly of the TCC on the cell surface results in cell lysis. Deposition of TCC on erythrocytes results in the destruction of these cells in hemolytic diseases such as PNH[13,14].

The importance of terminal complement components in immunity is demonstrated by individuals with genetic deficiencies of C5, C6, C7, C8 or C9, who show an increased incidence of infections with *N. meningitidis*, a bacterium that can cause meningitis[8]. Vaccines against *N. meningitidis* are recommended for terminal complement–deficient individuals, although breakthrough infections have been reported[15]. Interestingly, *N. meningitidis* infections in terminal complement–deficient people are typically less severe than in normal individuals, possibly due to the lack of TCC-mediated endotoxin release from the bacteria[15].

Targeted blockade of the complement cascade at C5. In the rational design of a therapeutic complement inhibitor, C5 is an attractive target[1]. Because C5 is common to all pathways of complement activation, blockade at this point stops the progression of the cascade regardless of the stimuli. In addition, prevention of C5 cleavage effectively blocks the generation of the potent proinflammatory molecule C5a and the cell lytic TCC. Importantly, C5 blockade preserves the critical immunoprotective and immunoregulatory functions of upstream components that culminate in C3b-mediated opsonization and immune complex clearance.

Development of eculizumab

To generate potent inhibitors of complement component C5, panels of murine anti-human C5 monoclonal antibodies were generated and initially screened for the ability to inhibit TCC-mediated lysis of antibody-sensitized chicken erythrocytes by human complement in a standard hemolytic assay[16]. The most potent candidates were cloned and purified, and functional activity was further characterized to identify anti-C5 antibodies that could also effectively block the generation of C5a. Of ~30,000 hybridomas screened, one monoclonal antibody (mAb), designated murine 5G1.1 (m5G1.1 mAb), was identified that effectively blocked both TCC-mediated hemolysis and the generation of C5a, at a 0.5:1 molar ratio of antibody to C5. Murine 5G1.1 mAb binding to human C5 mapped to the N-terminal region of the alpha chain.

Engineering. To reduce the potential for immunogenicity, we cloned and grafted the complementarity-determining regions of m5G1.1 mAb into human heavy and light chain antibody frameworks (**Fig. 2**)[16,17]. The human heavy-chain framework was derived from the H20C3H antibody variable region[18], which contained no amino acid changes relative to the original germline sequence. The human light-chain framework was derived from the L23 antibody variable region[19], which contained only one amino acid change from the original germline sequence; the germline amino acid residue was restored at this position. The use of purely germline framework acceptor sequences should further minimize potential immunogenicity of the humanized antibody, because the immune system of every individual should be tolerant to these sequences.

The use of antibodies as therapeutics in some clinical settings is complicated by effector functions of such antibodies that serve to activate complement and/or to bind to antibody receptors (Fc receptors) on inflammatory cells after they have engaged their target. To reduce the potential for eculizumab to elicit proinflammatory responses, we replaced the heavy-chain constant region of the parental antibody with components of both human IgG2 and IgG4 constant regions (**Fig. 2**)[20]. The human IgG2 antibody isotype does not bind Fc receptors[21,22], whereas the human IgG4 isotype does not activate the complement cascade[23,24]. The IgG2/IgG4 hybrid constant region of eculizumab includes the CH1 and hinge regions of human IgG2 fused to the CH2 and CH3 regions of human IgG4, and lacks the ability to bind Fc receptor and to activate complement[20]. To avoid the generation of an antigenic site during the fusion, we used a restriction endonuclease cleavage site common to both IgG2 and IgG4 to join the two constant regions. Indeed, a total of 31 amino acids flanking the fusion site are identical between IgG2 and IgG4.

Binding characteristics. The humanized monoclonal antibody eculizumab maintained its ability to block the cleavage of C5 during complement activation as the antibody potently inhibited both the generation of C5a and C5b-9–mediated human serum hemolytic activity[16]. Eculizumab is highly species-restricted, with minimal activity against any other primate or mammalian C5. No nonspecific or unexpected

Samsung Bioepis Exhibit No. 1002
Page 1169

binding of eculizumab to any human tissue examined in a tissue cross-reactivity study was observed. In addition, the affinity of eculizumab for human C5 (K_d = 120 pM) is similar to that of the parental m5G1.1-mAb, indicating that the binding properties of the antibody were not compromised during the humanization process.

The eculizumab pharmacokinetic profile was analyzed using a one-compartmental model. When administered by intravenous infusion, eculizumab has a half-life of 272 ± 82 h, and its distribution appears to be primarily limited to the vascular space. Eculizumab serum concentrations appear to reach steady state after ~57 days. At steady state, the eculizumab accumulation ratio (R_{acc}) was calculated to be 1.075, which is indicative of minimal accumulation over time. Pharmacodynamic activity correlates directly with eculizumab serum concentrations and maintenance of trough levels above 35 μg/ml results in essentially complete blockade of hemolytic activity *in vivo*[25].

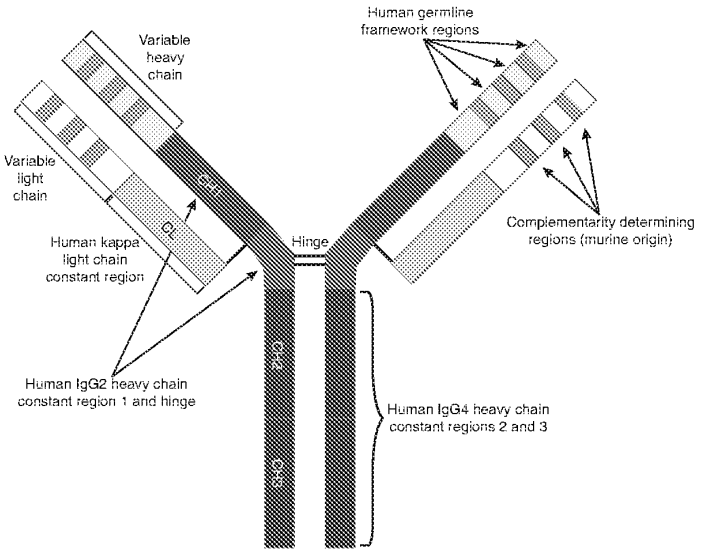


Figure 2 Engineering of eculizumab to reduce immunogenicity and eliminate effector functions. To minimize immunogenicity, we grafted murine complementarity-determining regions into human heavy and light chain germline antibody framework sequences. Additionally, human IgG2 and IgG4 heavy chain sequences were combined to form a hybrid constant region that is unable to bind Fc receptors or to activate the complement cascade. Eculizumab exhibits high affinity for human C5, effectively blocking its cleavage and downstream proinflammatory and cell lytic properties.

Preclinical development. Because of the species-restricted nature of eculizumab, pharmacological activities *in vivo* were explored with a surrogate anti-mouse C5 antibody (BB5.1) that demonstrated terminal complement–inhibitory activity comparable to that of eculizumab. As no adequate mouse models of PNH exist, *in vivo* studies targeting other therapeutic indications with potential involvement of terminal complement activation were used. In a collagen-induced model of arthritis, BB5.1 was shown to prevent the onset of disease and to improve the course of previously established disease[26]. Treatment with BB5.1 also resulted in an improvement in renal disease and in dramatic prolongation of survival in a lupus-like autoimmune model[27]. These models provided substantial evidence that a functionally blocking anti-C5 antibody could effectively and consistently block terminal complement activation leading to a reduction in terminal complement-mediated proinflammatory sequelae. The surrogate BB5.1 antibody was also used in mouse toxicity studies in which no compound-related mortalities or significant abnormalities were observed.

Early clinical development. Initial human clinical studies of eculizumab, and a single-chain antibody variant of eculizumab called pexelizumab, were performed in individuals with rheumatoid arthritis and systemic lupus erythematosus[28,29] and in individuals with coronary artery bypass graft surgery and myocardial infarction, respectively[30,31]. The initial eculizumab studies served two purposes, to determine a dosing schedule for future trials and to establish initial safety of terminal complement inhibition in humans. Participants received a single dose of eculizumab ranging from 0.1 mg/kg to 8 mg/kg; all doses were observed to be safe and well-tolerated. The 8 mg/kg dose (~600 mg total) provided complete complement blockade for 7–14 days. Studies exploring multiple chronic dose regimens with eculizumab were also performed in individuals with membranous nephritis and rheumatoid arthritis[32,33].

PNH—a disease in search of a complement inhibitor

Etiology of PNH. PNH is a rare form of hemolytic anemia with an estimated incidence of 1.3 cases per million per year and a prevalence of 15.9 cases per million[34]. The disease is manifest by an acquired genetic deficiency of endogenous complement inhibitors on the surface of blood cells; these cells are referred to as the PNH clone(s). PNH clones are deficient in proteins that are normally linked to the cell surface by a glycosylphosphatidylinositol (GPI)-anchor. The inability of cells to attach proteins through this lipid moiety is caused by somatic mutations occurring in the X-linked gene, phosphatidylinositol glycan–complementation class A (*PIGA*)[35–37], a critical molecule in the GPI-anchoring pathway.

In individuals with PNH, cells deficient in GPI-anchored proteins exist in all blood lineages including erythroid, myeloid and lymphoid cells. These PNH cells are clonal and harbor matching *PIGA* mutations indicating that the mutations occur in multipotent hematopoietic stem cells[38,39]. Granulocytes from the blood of healthy controls also possess *PIGA* mutations at an exceedingly low frequency (~1 in 50,000), suggesting that random mutations in this gene are important in the pathogenesis of PNH[40]. Thus, it has been hypothesized that the development of PNH requires two independent events to occur; hematopoietic stem cells must acquire a *PIGA* mutation and an immune attack that preferentially targets normal stem cells must be present[41,42]. This proposal presumes that the bone marrow insult targets a GPI-anchored protein on the hematopoietic stem cell, thereby providing a survival advantage to GPI-anchoring–deficient stem cells (that is, cells possessing a *PIGA* mutation). It should be noted however that *PIGA* mutations characterized in healthy controls appear to occur in more differentiated colony-forming cells rather than multipotent hematopoietic stem cells[39]. These colony-forming cells would not have self-renewal capacity and therefore would not be propagated beyond their terminally differentiated progeny.

There is clinical evidence to support a survival advantage of *PIGA* mutated hematopoietic stem cell clones over normal stem cells in damaged bone marrow. The mAb alemtuzumab (Campath) is used to treat

Samsung Bioepis Exhibit No. 1002
Page 1170

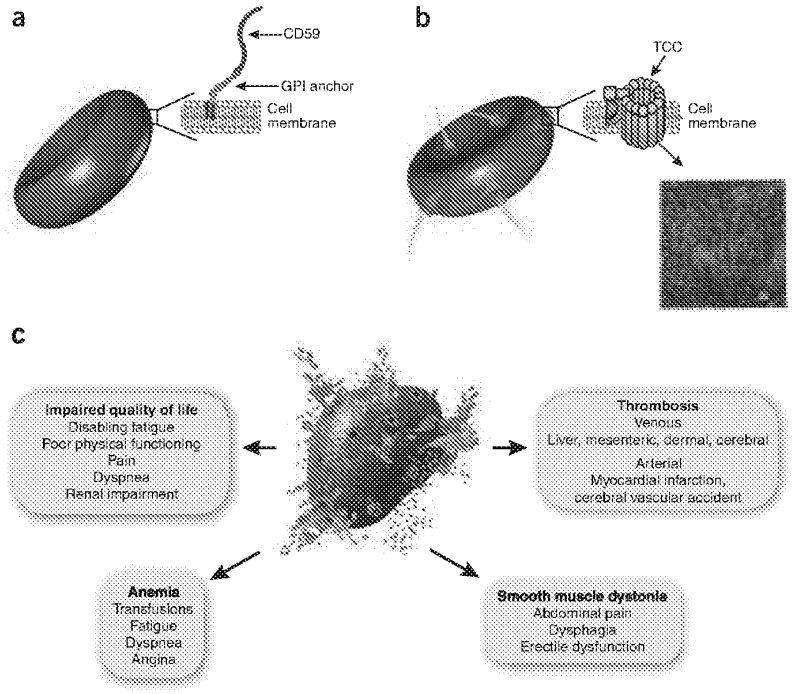


Figure 3 Terminal complement deposition on the surface of PNH RBCs results in chronic hemolysis and serious clinical morbidities. (**a**) Normal RBCs express complement regulatory proteins including the terminal complement inhibitor CD59. CD59 is tethered to the RBC lipid bilayer of the cell membrane by a GPI anchor. The presence of CD59 protects these cells from autologous complement-mediated destruction. (**b**) PNH RBCs lack GPI-anchored proteins including CD59. Continuous complement activation leads to formation of the terminal complement complex (TCC) that transverses the lipid bilayer. These complexes form numerous pores in the RBC membrane, as shown by the electron micrograph (inset). As pores form in the membrane, water enters the RBC (arrows), resulting from osmotic pressure. (**c**) As the concentration of TCCs increases, the PNH RBC swells and ultimately ruptures (hemolysis). Intravascular hemolysis causes the release of the RBC contents such as hemoglobin into the plasma. Hemolysis in PNH is chronic and leads to multiple clinical sequelae, including severe anemia requiring transfusions, disabling fatigue, dyspnea, impaired quality of life, recurrent pain associated with smooth muscle dystonia, and thrombosis. Artwork by Edmond Alexander, ©2007 Edmond Alexander, printed with permission. Micrograph image is from E.R. Podack. Molecular mechanisms of cytolysis by complement and cytolytic lymphocytes. *J. Cell. Biochem.* **30**, 133–170 (1986). Copyright © 1986 Wiley-Liss. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

chronic lymphocytic leukemia by targeting and destroying malignant T- and B-cells that express the GPI-anchored protein CD52. In many individuals, a PNH T-cell clone expands after alemtuzumab treatment. In this case alemtuzumab supplies the bone marrow insult and the GPI-anchoring deficient progenitor T-cells that lack CD52 escape immune destruction and expand[43].

Pathophysiology of PNH. Although PNH blood cells of all lineages are missing the wide array of GPI-anchored proteins from their surface, they are remarkably normal with regard to their physiological functions (e.g., fighting infection, clotting, carrying oxygen). However, the PNH red blood cell (RBC) is exquisitely sensitive to destruction because of the inability to regulate autologous terminal complement assembly (**Fig. 3**). This defect is primarily triggered by the absence of terminal complement inhibitor function[13,14,44,45]. CD59 normally blocks formation of the TCC on the RBC surface, thereby preventing intravascular hemolysis[46–48]. Hemolysis in PNH is chronic because of a continuous state of complement activation (tick-over; **Fig. 1**), but brisk episodes of hemolysis (paroxysms) coincident with increases in complement activation triggered by infections, surgery, strenuous exercise, excessive alcohol intake and blood transfusions frequently occur[49].

Chronic hemolysis is central to the morbidities in PNH and contributes to mortality in those with the disease (**Fig. 3**). It may result in severe anemia requiring transfusion, and refractory transfusion-dependent hemolytic anemia has been considered a major complication of PNH warranting consideration of bone marrow transplantation[35]. Fatigue in PNH patients is tied directly to hemolysis, strikingly disproportionate to the degree of anemia, frequently debilitating and similar to the severity observed in anemic cancer patients[25,49–52]. Hemolysis is also directly linked to diminished health status and functioning[25,49]. Other disabling morbidities evidenced by individuals with PNH that are likely a direct result of hemolysis include dysphagia, abdominal pain, erectile dysfunction, pulmonary hypertension and renal failure[49,53,54]. Thromboembolism, the most feared complication in PNH, is also directly associated with hemolysis and may be induced by the release of free hemoglobin, consumption of nitric oxide, and subsequent clotting[53,55–63].

Thrombosis is clinically evident in ~40% of individuals with PNH and is the leading cause of premature mortality[64–68]. Although venous thrombosis is more typical in PNH, arterial thrombosis, particularly in the cerebral circulation, is not uncommon[56,64]. Thrombosis is evident in individuals with small and large PNH clone sizes and varying degrees of hemolysis, although it appears to be more common in those with larger clones[56,57,66,68]. Individuals with hemolysis but without a history of transfusion also experience thrombosis, including fatal first thrombosis[57]. Subclinical thrombosis has been reported in 60% of a cohort of PNH patients, despite use of anticoagulation, and unrelated to transfusion requirements[69].

Complement inhibitors in PNH. As PNH is defined by the acquired genetic deficiency of the endogenous terminal complement inhibitor CD59 on the surface of blood cells, restoration of terminal complement inhibition should effectively reduce hemolysis in PNH and abrogate the serious clinical morbidities associated with this life-threatening disease. Initial attempts to circumvent the functional defect in PNH cells focused on replacing CD59 on the surface of PNH cells through gene therapy. A recombinant, transmembrane form of CD59 (CD59-TM) was generated and analyzed for the ability to regulate complement activity[70]. A GPI-anchoring deficient complement-sensitive B-cell line derived from an individual with PNH was virally transduced with CD59-TM, resulting in protection against classical complement-mediated membrane damage. These data established that a functional transmembrane form of CD59 can be expressed on the surface of PNH cells

Samsung Bioepis Exhibit No. 1002
Page 1171

through a gene therapy approach. Retroviral transduction of PNH erythroid cells with a functional *PIGA* gene has also been shown to restore GPI-anchor protein expression on the surface of these cells, thereby protecting them from complement-mediated destruction[71]. However, to provide a renewable source of complement-protected erythrocytes in individuals with PNH through a gene therapy approach, efficient targeting of early erythroid progenitor cells would be required, which has not been achieved to date. This led to the development of the complement inhibitor eculizumab, which binds to and blocks the cleavage of C5 in plasma, thereby reestablishing terminal complement regulation in the plasma of individuals with PNH. The potential effect of terminal complement blockade on hemolysis in PNH was observed in a person with coexistent C9 deficiency and PNH who had no signs of hemolysis[72]. PNH was diagnosed in this individual only after the administration of a whole blood transfusion during a routine surgery, which served to reconstitute C9 and trigger hemolysis.

Table 1 Efficacy results of the open-label phase 2 pilot study of eculizumab in individuals with PNH

	Before treatment	During eculizumab	*P* value
Units of packed RBCs transfused/patient/month			
Median	1.8	0	0.003
Mean ± s.e.m.	2.1 ± 0.83	0.6 ± 0.67	
LDH (mean ± s.e.m.; U/L)[a]	3111 ± 598	594 ± 32	0.002
PNH RBCs (proportions)	36.7 ± 5.9	59.2 ± 8.0	0.005
Days with hemoglobinuria/patient/month	2.9	0.12	<0.001

[a]The normal range for LDH in this study was 150–480 U/L.

Eculizumab in PNH: clinical trials

Eculizumab was evaluated in PNH in three separate parent studies—a phase 2 pilot study and two phase 3 studies. All participants in these trials were allowed to continue eculizumab in follow-on extension studies.

Pilot study. The first eculizumab PNH study (beginning in May 2002) evaluated terminal complement inhibition in a 3-month open-label phase 2 pilot study in 11 participants with hemolytic PNH at two sites in the United Kingdom[73]. In this study, participants with at least four transfusions in the previous 12 months received 600-mg infusions of eculizumab every week for 4 weeks, followed 1 week later by a 900-mg dose and then by 900 mg every other week through week 12. In this study and all subsequent trials, participants were vaccinated against *N. meningitidis* at least 2 weeks before the first dose of eculizumab. Clinical and biochemical indicators of hemolysis were measured throughout the trial. Mean lactate dehydrogenase (LDH) levels decreased from 3111 U per liter before treatment to 594 U per liter during treatment ($P = 0.002$; **Table 1**). The mean percentage of PNH erythrocytes increased from 36.7% of the total erythrocyte population to 59.2% ($P = 0.005$). The mean and median transfusion rates decreased from 2.1 and 1.8 units per individual per month to 0.6 and 0.0 units per individual per month, respectively ($P = 0.003$, for the comparison of the median rates). Episodes of hemoglobinuria were reduced by 96% ($P < 0.001$), and measurements of the quality of life substantially improved. From this study, it appeared that eculizumab was safe and well-tolerated in individuals with PNH. This terminal complement inhibitor reduced hemolysis, hemoglobinuria, and the need for transfusion, with an associated improvement in quality of life.

After completion of the initial 12-week study, all 11 participants from the initial PNH study chose to continue treatment in a 52-week extension study; all participants completed the study[25]. The dramatic reductions in hemolysis and the need for transfusion that were observed in the pilot study were maintained throughout the extension study, and significant improvements in quality-of-life measures also continued. In this study, long-term eculizumab administration continued to be safe and well-tolerated. Clinical breakthrough occurred in two individuals with insufficient eculizumab levels; shortening the dosing interval (increasing the total dose) resulted in rapid restoration of complement suppression, abrogation of hemolysis, and resolution of disease signs and symptoms. These data demonstrate the close relationship between sustained terminal complement inhibition, hemolysis, and symptoms in individuals with PNH. Further, dramatic clinical improvements in signs and symptoms attributed specifically to hemolysis including hemoglobinuria, and the smooth muscle dystonias—abdominal pain, dysphagia and erectile dysfunction—were observed in these participants[54]. Ten of the original 11 participants have remained on eculizumab for more than 5 years of continuous treatment.

Phase 3 pivotal program. The results from the initial open-label phase 2 pilot study were sufficiently compelling that discussions were initiated with the FDA and the EMEA in order to plan phase 3 studies for registration in the United States and Europe, respectively. In 2003,

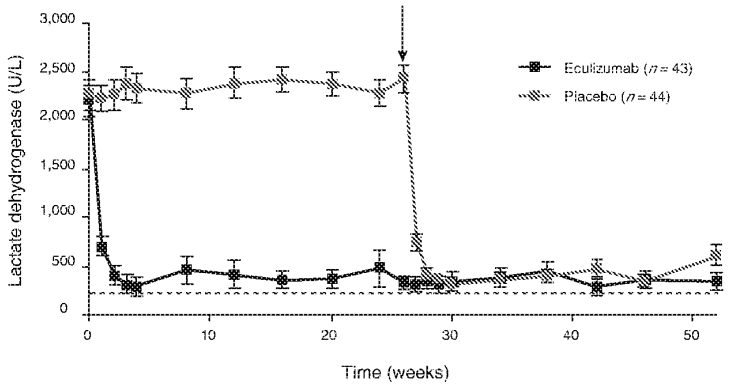


Figure 4 Reduction in hemolysis during treatment with eculizumab. Mean levels of lactate dehydrogenase reflect the degree of hemolysis from baseline to week 52. The dashed line indicates the upper limit of the normal range for lactate dehydrogenase (normal range, 103 to 223 U per liter). In eculizumab-treated patients, the mean level of lactate dehydrogenase was rapidly reduced to just above the upper limit of the normal range. In the placebo group, the mean level of lactate dehydrogenase remained highly elevated. The arrow depicts the transition of placebo-treated patients in TRIUMPH to eculizumab treatment in the phase 3 extension study at which time levels of lactate dehydrogenase rapidly reduced to near normal values.

Samsung Bioepis Exhibit No. 1002
Page 1172

Table 2 Primary and secondary endpoints of the phase 3 pivotal efficacy study TRIUMPH

	Treatment period		
	Placebo	Eculizumab	*P* value
Stabilization of hemoglobin levels (percent of patients)[a]	0	49	<0.000001
Units transfused/patient[a]			
Median	10	0	<0.000001
Mean ± s.e.m.	11.0 ± 0.83	3.0 ± 0.67	
Transfusion Avoidance (percent of patients)	0	51	<0.000001
FACIT-Fatigue score (change from baseline)	−4.0 ± 1.7	6.4 ± 1.2	0.000005
LDH (area under the curve; U/L × day)	411,822	58,587	<0.000001

[a]Co-primary endpoints.

eculizumab received orphan designation for PNH in both the United States and Europe. The phase 3 eculizumab in PNH program consisted of: TRIUMPH, the pivotal trial that examined eculizumab safety and efficacy in a hemolytic PNH population with at least 4 transfusions in 12 months; SHEPHERD, which examined safety and efficacy of eculizumab in a broader and more heterogeneous hemolytic PNH population in individuals with a history of transfusion; and a phase 3b extension study to evaluate long-term safety and efficacy, including the potential anti-thrombotic effect of eculizumab.

TRIUMPH was a double-blind, randomized, placebo-controlled, multicenter, phase 3 trial[74]. Individuals were first enrolled and treated in 2004 and received either placebo or eculizumab intravenously; eculizumab was given at a dose of 600 mg weekly for 4 weeks, followed 1 week later by a 900-mg dose and then 900 mg every 14 ± 2 days through week 26. Eighty-seven participants (43 eculizumab, 44 placebo) at 34 international sites were enrolled. The two primary endpoints were the stabilization of hemoglobin levels and the number of units of packed red cells transfused. Biochemical indicators of hemolysis and the participant's fatigue and quality of life were also assessed. After enrollment, participants were observed for up to 13 weeks. Those who did not require a transfusion during the observation period were considered ineligible for randomization. Eighty-seven individuals underwent randomization. Every participant treated with eculizumab experienced an objective response. Across all treated participants, a significant reduction in hemolysis (as measured by LDH) was observed after 1 week of treatment and maintained throughout the 26 week study in the eculizumab group but not in placebo (**Fig. 4**). There was an 85.8% lower median area under the curve for LDH plotted against time (in days) in the eculizumab group, as compared with the placebo group (58,587 versus 411,822 U per liter, respectively; $P < 1 \times 10^{-6}$; **Table 2**). After completion of the study, a similar reduction in hemolysis was demonstrated with placebo participants transitioning to eculizumab. Stabilization of hemoglobin levels in the absence of transfusions was achieved in 49% (21 of 43) of the individuals assigned to eculizumab and none (0 of 44) of those assigned to placebo ($P < 1 \times 10^{-6}$). During the study, a median of 0 units of packed red cells was administered in the eculizumab group, as compared with 10 units in the placebo group ($P < 1 \times 10^{-6}$). A total of 51% of the eculizumab group remained transfusion independent during the entire 26-week study, whereas no one in the placebo group achieved this status. Even among participants receiving eculizumab in whom transfusion independence was not achieved, transfusion requirements were reduced by 44% as these individuals also demonstrated a clinically and statistically significant reduction in hemolysis. The reduced but continued requirement for transfusion support in some individuals may reflect more severe bone marrow aplasia and/or residual low-level hemolysis through the extravascular compartment[74]. Clinically and statistically significant improvements were also found in fatigue, as measured by scores on the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-Fatigue) instrument ($P = 6 \times 10^{-6}$) and the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30; $P < 1 \times 10^{-6}$). Participants that did not achieve complete transfusion independence during eculizumab treatment also experienced significant improvement in fatigue and other measures of quality of life. Of the 87 participants, 4 in the eculizumab group and 9 in the placebo group had serious adverse events, none of which were considered to be treatment-related; all these patients recovered without sequelae. The most common adverse events reported in the eculizumab group were headache, nasopharyngitis, back pain and nausea. Headache and back pain occurred more frequently in the eculizumab group than in the placebo group. Anti-eculizumab antibody responses were of low titer, transient, and occurred at a frequency similar to that of placebo. The TRIUMPH study demonstrated that eculizumab is a well-tolerated and effective therapy for PNH.

After initiation of the TRIUMPH study, eculizumab was evaluated in SHEPHERD, an open-label, non-placebo controlled, phase 3 clinical safety and efficacy study in a more diverse PNH population including patients with marked thrombocytopenia and minimal transfusion requirements. Participants were first enrolled and treated in 2005, and eculizumab was administered for a total of 52 weeks using a dosing schedule identical to that of TRIUMPH. Ninety-seven individuals at 33 international sites were enrolled. Adverse events were similar to those with placebo in the TRIUMPH study. Hemolysis was significantly reduced leading to improved anemia with a reduction in or elimination of transfusion requirement, lessened fatigue and improved overall health-related quality of life[75]. Compared to the overall population, efficacy with eculizumab was similar in individuals who would be expected to have less severe disease, including patient subgroups with lower baseline hemolysis, mild anemia, and minimal or no pretreatment transfusion requirement[76].

Of the 195 individuals entering the pilot, TRIUMPH or SHEPHERD studies, 187 participants completed these studies and elected to receive eculizumab in a common 104-week phase 3b open-label extension study. Overall, there was no evidence of an increased incidence of infection across PNH studies with eculizumab as compared to placebo, including serious infections, severe infections or multiple infections. Two individuals vaccinated against *N. meningitidis* experienced meningococcal sepsis; they were treated and recovered without clinical sequelae. Serious hemolysis was not observed in any of the initial 16 participants who discontinued treatment. Long-term eculizumab treatment was associated with a reduction in hemolysis up to more than 54 months of continuous treatment. Importantly, the design of the phase 3b extension study specified that the thrombosis rate for each participant would be compared before and during eculizumab treatment. Early evaluation shows that the 7.37 thrombotic events per 100 patient-years experienced before eculizumab was reduced to 1.07 events per 100 patient-years with eculizumab treatment ($P < 1 \times 10^{-6}$)[77].

Approval of eculizumab. After completion of TRIUMPH and after the pre-specified interim 26-week analysis in SHEPHERD, applications for marketing authorization of eculizumab in the treatment of PNH were submitted to the FDA and EMEA in September 2006. The FDA

Samsung Bioepis Exhibit No. 1002
Page 1173

accepted the application for priority review and in March 2007, less than 6 months after the completion of SHEPHERD, FDA approved eculizumab for the treatment of patients with PNH to reduce hemolysis. The EMEA granted review of the eculizumab application under the Accelerated Assessment procedure, the fastest evaluation time frame for full approval awarded by EMEA. In June 2007, eculizumab became the first medicinal product to receive approval by the European Commission within the Accelerated Assessment procedure and it is indicated for the treatment of patients with PNH.

Conclusions and perspectives

Clinical development of eculizumab spanned a full decade before its first approval in the rare, disabling and life-threatening, clonal blood disorder PNH (**Fig. 5**). The studies before initiation of the PNH clinical program provided an important assessment of the safety profile and immunomodulatory effects of eculizumab. Once initiated, the PNH program has been one of the most extensive pre-approval clinical programs for an 'ultra-orphan' disease; the design of the multinational trials enrolling 195 participants allowed sequential, controlled and long-term evaluation of both biochemical and clinically validated measurements in the PNH population. This paradigm of careful evaluation may be used in the consideration of eculizumab in other uncommon, debilitating and life-threatening diseases for which there is evidence suggesting that terminal complement may significantly contribute to the pathophysiology of the disease, including antibody-mediated transplant rejection[78], myasthenia gravis and other peripheral neuropathies[79–83], fulminant lupus[27,29], catastrophic anti-phospholipid syndrome[84,85] and pattern 2 multiple sclerosis[86]. The understanding of critical pharmacokinetic and pharmacodynamic properties in PNH patients and the accomplishment of large-scale commercial manufacturing of eculizumab may also permit the evaluation of new formulations of eculizumab in future development programs for still other diseases with evidence suggesting complement-mediated pathology, including asthma[87] and age-related macular degeneration[88].

Clinical trials have demonstrated that eculizumab dramatically and reproducibly abrogates intravascular hemolysis, the primary clinical manifestation of PNH. These trials have shown that the reduction in hemolysis is independently associated with improvements in major morbidities in individuals with PNH including anemia, fatigue and health-related quality of life, as well as fewer thrombotic events. Because of the extremely rare nature of the disease and the ethical dilemma posed by the efficacy evident with eculizumab in the treatment of PNH, a prospective randomized controlled clinical trial is not a feasible means to examine the long-term impact of eculizumab on survival in individuals with PNH. However, given the evident anti-hemolytic effect and attendant clinical benefit of eculizumab administration, it is possible that eculizumab could beneficially impact several of the leading causes of premature mortality in PNH including venous and arterial thrombosis, hemorrhage that may be worsened by platelet consumption, renal failure and liver failure. In a *post hoc* analysis of the survival of eculizumab-treated PNH patients compared to historical controls[64], ~15 deaths would have been expected during the 2-year period of eculizumab treatment; however, two deaths were observed during this period, or 87% fewer than expected ($P = 0.0005$). This preliminary analysis suggests that long-term eculizumab treatment may beneficially affect survival in individuals diagnosed with PNH.

The humanization strategy used to construct eculizumab has resulted in only very rare evidence of responses to eculizumab, with an incidence similar to that found with placebo treatment. There is no evidence of tolerance or a neutralizing antibody response in any treated individual to date and infusion-related immune effects are similar in frequency to placebo. It is interesting to speculate that the selection of purely germline framework acceptor regions, seen as native by all recipients, has allowed for this important safety benefit.

Rigorous evaluation of eculizumab in PNH has helped tease out the role of hemolysis in mediating critical patient-reported outcomes, including fatigue and patient functioning. The reproducible demonstration that patient improvement with eculizumab, due to the abrogation

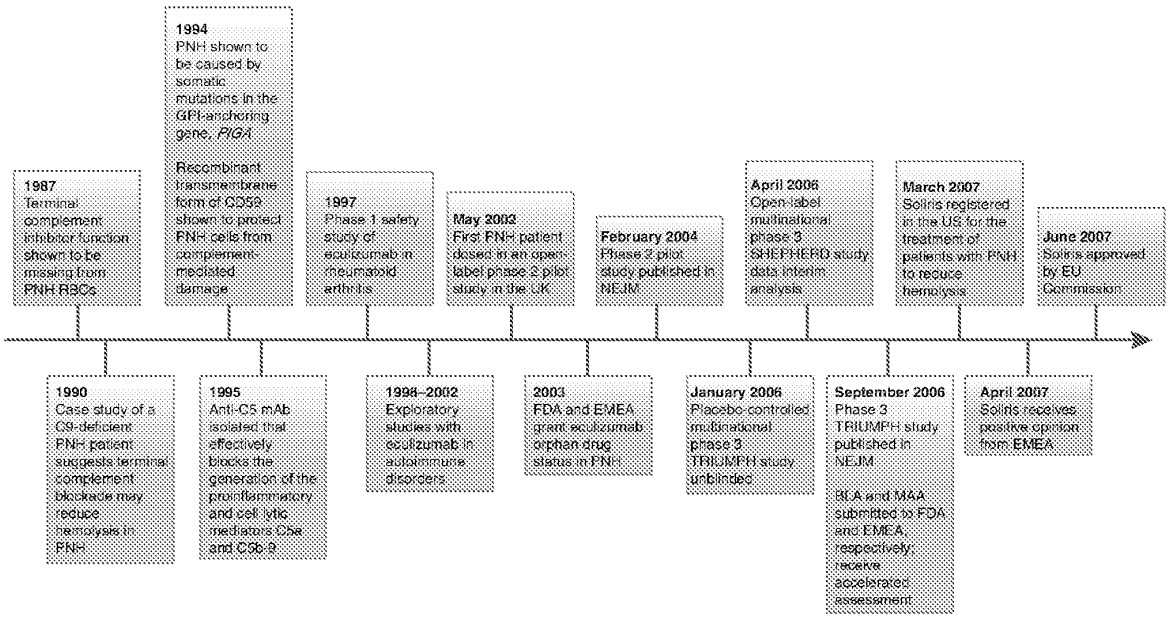


Figure 5 Eculizumab in PNH development timeline. BLA, biologic license application; MAA, marketing authorization application.

Samsung Bioepis Exhibit No. 1002
Page 1174

of the underlying hemolysis, is distinct from associated improvements in anemia may have applications to understanding the potential multifactorial etiology of fatigue in individuals with anemia in other disease settings, such as cancer. Similarly, evident improvement in smooth muscle dystonias and thrombosis with eculizumab treatment in PNH has helped to establish new and important clinical paradigms, particularly with regard to the role of cell-free plasma hemoglobin and nitric oxide scavenging in thrombosis, smooth muscle dystonia and vasomotor abnormalities[53]. In this case, focus on a rare disease may not only lead to benefit for afflicted individuals but may also contribute to the overall foundation of knowledge. Such knowledge may provide insight into more common diseases with disordered nitric oxide regulation, including systemic and pulmonary hypertension, diabetes, sickle cell disease, and other genetic or acquired hemolytic anemias.

In summary, data from six clinical studies, enrolling a total of 195 participants, demonstrate the robust efficacy of eculizumab for the treatment of individuals with PNH. Every individual treated with eculizumab to date has shown an objective improvement in hemolysis. Treatment with eculizumab modifies the clinical course of PNH and markedly reduces the serious morbidities associated with the disease. In particular, therapy with eculizumab reduces anemia, blood transfusion requirements, and the risk for life-threatening thrombosis. Eculizumab also improves other morbidities that are often serious, including fatigue, pain, dyspnea and impaired overall quality of life. Treated individuals will continue to be monitored in a global registry. Overall, the therapeutic benefit and safety of eculizumab in the treatment of patients with the rare blood disease PNH validates the utility of terminal complement inhibitor therapeutics for the treatment of medical conditions where complement activation is involved.

ACKNOWLEDGMENTS

The authors wish to acknowledge the Alexion research team for their involvement in the identification and characterization of eculizumab, the Alexion clinical and drug development teams for the eculizumab clinical program, the clinicians and patients for their participation in the trials, and Kerry Quinn-Senger and Rebecca Baker for assistance with the preparation of this manuscript.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturebiotechnology/.

Published online at http://www.nature.com/naturebiotechnology/
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions

1. Matis, L.A. & Rollins, S.A. Complement-specific antibodies: designing novel anti-inflammatories. *Nat. Med.* **1**, 839–842 (1995).
2. Walport, M.J. Complement. Second of two parts. *N. Engl. J. Med.* **344**, 1140–1144 (2001).
3. Male, D., Brostoff, J., Roth, D. & Roitt, I. *Immunology* (Mosby-Elsevier, Philadelphia, 2006).
4. Abdul Ajees, A. *et al.* The structure of complement C3b provides insights into complement activation and regulation. *Nature* **444**, 221–225 (2006).
5. Janssen, B.J. *et al.* Structures of complement component C3 provide insights into the function and evolution of immunity. *Nature* **437**, 505–511 (2005).
6. Janssen, B.J., Christodoulidou, A., McCarthy, A., Lambris, J.D. & Gros, P. Structure of C3b reveals conformational changes that underlie complement activity. *Nature* **444**, 213–216 (2006).
7. Wiesmann, C. *et al.* Structure of C3b in complex with CRIg gives insights into regulation of complement activation. *Nature* **444**, 217–220 (2006).
8. Overturf, G.D. Indications for the immunological evaluation of patients with meningitis. *Clin. Infect. Dis.* **36**, 189–194 (2003).
9. Huber-Lang, M. *et al.* Generation of C5a in the absence of C3: a new complement activation pathway. *Nat. Med.* **12**, 682–687 (2006).
10. Muller-Eberhard, H.J. Molecular organization and function of the complement system. *Annu. Rev. Biochem.* **57**, 321–347 (1988).
11. Rabiet, M.J., Huet, E. & Boulay, F. The N-formyl peptide receptors and the anaphylatoxin C5a receptors: an overview. *Biochimie* **89**, 1089–1106 (2007).
12. Chen, N.J. *et al.* C5L2 is critical for the biological activities of the anaphylatoxins C5a and C3a. *Nature* **446**, 203–207 (2007).
13. Yamashina, M. *et al.* Inherited complete deficiency of 20-kilodalton homologous restriction factor (CD59) as a cause of paroxysmal nocturnal hemoglobinuria. *N. Engl. J. Med.* **323**, 1184–1189 (1990).
14. Motoyama, N., Okada, N., Yamashina, M. & Okada, H. Paroxysmal nocturnal hemoglobinuria due to hereditary nucleotide deletion in the HRF20 (CD59) gene. *Eur. J. Immunol.* **22**, 2669–2673 (1992).
15. Platonov, A.E., Vershinina, I.V., Kuijper, E.J., Borrow, R. & Kayhty, H. Long term effects of vaccination of patients deficient in a late complement component with a tetravalent meningococcal polysaccharide vaccine. *Vaccine* **21**, 4437–4447 (2003).
16. Thomas, T.C. *et al.* Inhibition of complement activity by humanized anti-C5 antibody and single-chain Fv. *Mol. Immunol.* **33**, 1389–1401 (1996).
17. Riechmann, L., Clark, M., Waldmann, H. & Winter, G. Reshaping human antibodies for therapy. *Nature* **332**, 323–327 (1988).
18. Weng, N.P., Yu-Lee, L.Y., Sanz, I., Patten, B.M. & Marcus, D.M. Structure and specificities of anti-ganglioside autoantibodies associated with motor neuropathies. *J. Immunol.* **149**, 2518–2529 (1992).
19. Klein, R., Jaenichen, R. & Zachau, H.G. Expressed human immunoglobulin kappa genes and their hypermutation. *Eur. J. Immunol.* **23**, 3248–3262 (1993).
20. Mueller, J.P. *et al.* Humanized porcine VCAM-specific monoclonal antibodies with chimeric IgG2/G4 constant regions block human leukocyte binding to porcine endothelial cells. *Mol. Immunol.* **34**, 441–452 (1997).
21. Canfield, S.M. & Morrison, S.L. The binding affinity of human IgG for its high affinity Fc receptor is determined by multiple amino acids in the CH2 domain and is modulated by the hinge region. *J. Exp. Med.* **173**, 1483–1491 (1991).
22. Burton, D.R. & Woof, J.M. Human antibody effector function. *Adv. Immunol.* **51**, 1–84 (1992).
23. Tao, M.H., Smith, R.I. & Morrison, S.L. Structural features of human immunoglobulin G that determine isotype-specific differences in complement activation. *J. Exp. Med.* **178**, 661–667 (1993).
24. Smith, R.I., Coloma, M.J. & Morrison, S.L. Addition of a mu-tailpiece to IgG results in polymeric antibodies with enhanced effector functions including complement-mediated cytolysis by IgG4. *J. Immunol.* **154**, 2226–2236 (1995).
25. Hill, A. *et al.* Sustained response and long-term safety of eculizumab in paroxysmal nocturnal hemoglobinuria. *Blood* **106**, 2559–2565 (2005).
26. Wang, Y., Rollins, S.A., Madri, J.A. & Matis, L.A. Anti-C5 monoclonal antibody therapy prevents collagen-induced arthritis and ameliorates established disease. *Proc. Natl. Acad. Sci. USA* **92**, 8955–8959 (1995).
27. Wang, Y. *et al.* Amelioration of lupus-like autoimmune disease in NZB/WF1 mice after treatment with a blocking monoclonal antibody specific for complement component C5. *Proc. Natl. Acad. Sci. USA* **93**, 8563–8568 (1996).
28. Jain, R.I., Moreland, L.W., Caldwell, J.R., Rollins, S.A. & Mojcik, C.F. A single dose, placebo controlled, double blind, phase I study of the humanized anti-C5 antibody h5G1.1 in patients with rheumatoid arthritis. *Arthritis Rheum.* **42**, S77 (1999).
29. Rother, R.P., Mojcik, C.F. & McCroskery, E.W. Inhibition of terminal complement: a novel therapeutic approach for the treatment of systemic lupus erythematosus. *Lupus* **13**, 328–334 (2004).
30. Verrier, E.D. *et al.* Terminal complement blockade with pexelizumab during coronary artery bypass graft surgery requiring cardiopulmonary bypass: a randomized trial. *J. Am. Med. Assoc.* **291**, 2319–2327 (2004).
31. Armstrong, P.W. *et al.* Pexelizumab for acute ST-elevation myocardial infarction in patients undergoing primary percutaneous coronary intervention: a randomized controlled trial. *J. Am. Med. Assoc.* **297**, 43–51 (2007).
32. Tesser, J. *et al.* Safety and efficacy of the humanized anti-C5 antibody h5G1.1 in patients with rheumatoid arthritis. *Arthritis Rheum.* **44**, S274 (2001).
33. Appel, G. *et al.* Eculizumab (C5 complement inhibitor) in the treatment of idiopathic membranous nephropathy (IMN): Preliminary baseline and pharmacokinetic (PK) / Pharmacodynamic (PD) Data. *J. Am. Soc. Nephrol.* **13**, 668a (2002).
34. Hill, A. *et al.* The incidence and prevalence of paroxysmal nocturnal hemoglobinuria (PNH) and survival of patients in Yorkshire. *Blood* **108**, 985 (2006).
35. Parker, C. *et al.* Diagnosis and management of paroxysmal nocturnal hemoglobinuria. *Blood* **106**, 3699–3709 (2005).
36. Takeda, J. *et al.* Deficiency of the GPI anchor caused by a somatic mutation of the PIG-A gene in paroxysmal nocturnal hemoglobinuria. *Cell* **73**, 703–711 (1993).
37. Bessler, M. *et al.* Paroxysmal nocturnal haemoglobinuria (PNH) is caused by somatic mutations in the PIG-A gene. *EMBO J.* **13**, 110–117 (1994).
38. Oni, S.B., Osunkoya, B.O. & Luzzatto, L. Paroxysmal nocturnal hemoglobinuria: evidence for monoclonal origin of abnormal red cells. *Blood* **36**, 145–152 (1970).
39. Hu, R. *et al.* PIG-A mutations in normal hematopoiesis. *Blood* **105**, 3848–3854 (2005).
40. Araten, D.J., Nafa, K., Pakdeesuwan, K. & Luzzatto, L. Clonal populations of hematopoietic cells with paroxysmal nocturnal hemoglobinuria genotype and phenotype are present in normal individuals. *Proc. Natl. Acad. Sci. USA* **96**, 5209–5214 (1999).
41. Dacie, J. [Paroxysmal nocturnal haemoglobinuria.] *Sangre (Barc.)* **25**, 890–895 (1980).
42. Rotoli, B. & Luzzatto, L. Paroxysmal nocturnal hemoglobinuria. *Semin. Hematol.* **26**, 201–207 (1989).
43. Rawstron, A.C. *et al.* The PNH phenotype cells that emerge in most patients after CAMPATH-1H therapy are present prior to treatment. *Br. J. Haematol.* **107**, 148–153 (1999).
44. Hansch, G.M., Schonermark, S. & Roelcke, D. Paroxysmal nocturnal hemoglobinuria type III. Lack of an erythrocyte membrane protein restricting the lysis by C5b-9. *J. Clin. Invest.* **80**, 7–12 (1987).
45. Okada, N., Harada, R. & Okada, H. Erythrocytes of patients with paroxysmal nocturnal

Samsung Bioepis Exhibit No. 1002
Page 1175

hemoglobinuria acquire resistance to complement attack by purified 20-kD homologous restriction factor. *Clin. Exp. Immunol.* **80**, 109–113 (1990).
45. Holguin, M.H., Fredrick, L.R., Bernshaw, N.J., Wilcox, L.A. & Parker, C.J. Isolation and characterization of a membrane protein from normal human erythrocytes that inhibits reactive lysis of the erythrocytes of paroxysmal nocturnal hemoglobinuria. *J. Clin. Invest.* **84**, 7–17 (1989).
47. Rollins, S.A. & Sims, P.J. The complement-inhibitory activity of CD59 resides in its capacity to block incorporation of C9 into membrane C5b-9. *J. Immunol.* **144**, 3478–3483 (1990).
48. Rosenfeld, S.I., Jenkins, D.E., Jr & Leddy, J.P. Enhanced reactive lysis of paroxysmal nocturnal hemoglobinuria erythrocytes. Studies on C9 binding and incorporation into high molecular weight complexes. *J. Exp. Med.* **164**, 981–997 (1986).
49. Brodsky, R.A. Paroxysmal nocturnal hemoglobinuria. in *Hematology: Basic Principles and Practice* (eds. Hoffman, R. & Benz E. J. Jr. *et al.*) 419–427 (Churchill Livingstone, Philadelphia, 2005).
50. Bottomley, A. *et al.* Health-related quality of life in survivors of locally advanced breast cancer: an international randomised controlled phase III trial. *Lancet Oncol.* **6**, 287–294 (2005).
51. Cella, D., Lai, J.S., Chang, C.H., Peterman, A. & Slavin, M. Fatigue in cancer patients compared with fatigue in the general United States population. *Cancer* **94**, 528–538 (2002).
52. Wisloff, F., Gulbrandsen, N., Hjorth, M., Lenhoff, S. & Fayers, P. Quality of life may be affected more by disease parameters and response to therapy than by haemoglobin changes. *Eur. J. Haematol.* **75**, 293–298 (2005).
53. Rother, R.P., Bell, L., Hillmen, P. & Gladwin, M.T. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. *J. Am. Med. Assoc.* Review article correlating intravascular hemolysis and cell-free plasma hemoglobin to the clinical sequelae of PNH and other hemolytic diseases **293**, 1653–1662 (2005).
54. Hill, A., Rother, R.P. & Hillmen, P. Improvement in the symptoms of smooth muscle dystonia during eculizumab therapy in paroxysmal nocturnal hemoglobinuria. *Haematologica* **90**, ECR40 (2005).
55. Gralnick, H.R. *et al.* Activated platelets in paroxysmal nocturnal haemoglobinuria. *Br. J. Haematol.* **91**, 697–702 (1995).
56. Audebert, H.J., Planck, J., Eisenburg, M., Schrezenmeier, H. & Haberl, R.L. Cerebral ischemic infarction in paroxysmal nocturnal hemoglobinuria report of 2 cases and updated review of 7 previously published patients. *J. Neurol.* **252**, 1379–1386 (2005).
57. Hall, C., Richards, S. & Hillmen, P. Primary prophylaxis with warfarin prevents thrombosis in paroxysmal nocturnal hemoglobinuria (PNH). *Blood* **102**, 3587–3591 (2003).
58. Olsen, S.B. *et al.* Enhancement of platelet deposition by cross-linked hemoglobin in a rat carotid endarterectomy model. *Circulation* **93**, 327–332 (1996).
59. Radomski, M.W., Palmer, R.M. & Moncada, S. Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. *Lancet* **2**, 1057–1058 (1987).
60. Radomski, M.W., Palmer, R.M. & Moncada, S. The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. *Br. J. Pharmacol.* **92**, 639–646 (1987).
61. Shao, J. *et al.* Protective role of nitric oxide in a model of thrombotic microangiopathy in rats. *J. Am. Soc. Nephrol.* **12**, 2088–2097 (2001).
62. Catani, M.V., Bernassola, F., Rossi, A. & Melino, G. Inhibition of clotting factor XIII activity by nitric oxide. *Biochem. Biophys. Res. Commun.* **249**, 275–278 (1998).
63. Kayanoki, Y. *et al.* Reduced nitric oxide production by L-arginine deficiency in lysinuric protein intolerance exacerbates intravascular coagulation. *Metabolism* **48**, 1136–1140 (1999).
64. Hillmen, P., Lewis, S.M., Bessler, M., Luzzatto, L. & Dacie, J.V. Natural history of paroxysmal nocturnal hemoglobinuria. *N. Engl. J. Med.* **333**, 1253–1258 (1995).
65. Socie, G. *et al.* Paroxysmal nocturnal haemoglobinuria: long-term follow-up and prognostic factors. French Society of Haematology. *Lancet* **348**, 573–577 (1996).
66. Moyo, V.M., Mukhina, G.L., Garrett, E.S. & Brodsky, R.A. Natural history of paroxysmal nocturnal haemoglobinuria using modern diagnostic assays. *Br. J. Haematol.* **126**, 133–138 (2004).
67. Araten, D.J., Thaler, H.T. & Luzzatto, L. High incidence of thrombosis in African-American and Latin-American patients with paroxysmal nocturnal haemoglobinuria. *Thromb. Haemost.* **93**, 88–91 (2005).
68. Nishimura, J. *et al.* Clinical course and flow cytometric analysis of paroxysmal nocturnal hemoglobinuria in the United States and Japan. *Medicine (Baltimore)* **83**, 193–207 (2004).
69. Hill, A. *et al.* High definition contrast-enhanced MR imaging in paroxysmal nocturnal hemoglobinuria (PNH) suggests a high frequency of subclinical thrombosis. *Blood* **108**, 979 (2006).
70. Rother, R.P. *et al.* Expression of recombinant transmembrane CD59 in paroxysmal nocturnal hemoglobinuria B cells confers resistance to human complement. *Blood* **84**, 2604–2611 (1994).
71. Nishimura, J. *et al.* Efficient retrovirus-mediated PIG-A gene transfer and stable restoration of GPI-anchored protein expression in cells with PNH phenotype. *Blood* **97**, 3004–3010 (2001).
72. Yonemura, Y. *et al.* Paroxysmal nocturnal haemoglobinuria with coexisting deficiency of the ninth component of complement: lack of massive haemolytic attack. *Br. J. Haematol.* **74**, 108–113 (1990).
73. Hillmen, P. *et al.* Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. *N. Engl. J. Med.* **350**, 552–559 (2004).
74. Hillmen, P. *et al.* The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N. Engl. J. Med.* **355**, 1233–1243 (2006).
75. Schrezenmeier, H. *et al.* Safety and efficacy of the terminal complement inhibitor eculizumab in patients with paroxysmal nocturnal hemoglobinuria: SHEPHERD phase III clinical study results. *Haematologica* **92**, 1696 (2007).
76. Muus, P. *et al.* The clinical benefit of eculizumab is demonstrable in all subpopulations of patients with paroxysmal nocturnal hemoglobinuria (PNH) with hemolysis. *Haematologica* **92**, 379 (2007).
77. Hillmen, P. *et al.* Effect of the complement inhibitor eculizumab on thromboembolism in patients with paroxysmal nocturnal hemoglobinuria. *Blood*, published online 16 August 2007 (doi 10.1182/blood-2007-1106-095646).
78. Wang, H. *et al.* Prevention of acute vascular rejection by a functionally blocking anti-C5 monoclonal antibody combined with cyclosporine. *Transplantation* **79**, 1121–1127 (2005).
79. Biesecker, G. & Gomez, C.M. Inhibition of acute passive transfer experimental autoimmune myasthenia gravis with Fab antibody to complement C6. *J. Immunol.* **142**, 2654–2659 (1989).
80. Christadoss, P. C5 gene influences the development of murine myasthenia gravis. *J. Immunol.* **140**, 2589–2592 (1988).
81. Morgan, B.P. *et al.* The membrane attack pathway of complement drives pathology in passively induced experimental autoimmune myasthenia gravis in mice. *Clin. Exp. Immunol.* **146**, 294–302 (2006).
82. Sawant-Mane, S., Clark, M.B. & Koski, C.L. In vitro demyelination by serum antibody from patients with Guillain-Barre syndrome requires terminal complement complexes. *Ann. Neurol.* **29**, 397–404 (1991).
83. Willison, H.J. Basic and clinical aspects of autoimmune disorders in peripheral nerves. *Acta Neurol. Scand. Suppl.* **183**, 14–18 (2006).
84. Girardi, G. *et al.* Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome. *J. Clin. Invest.* **112**, 1644–1654 (2003).
85. Ritis, K. *et al.* A novel C5a receptor-tissue factor cross-talk in neutrophils links innate immunity to coagulation pathways. *J. Immunol.* **177**, 4794–4802 (2006).
86. Keegan, M. *et al.* Relation between humoral pathological changes in multiple sclerosis and response to therapeutic plasma exchange. *Lancet* **366**, 579–582 (2005).
87. Peng, T. *et al.* Role of C5 in the development of airway inflammation, airway hyperresponsiveness, and ongoing airway response. *J. Clin. Invest.* **115**, 1590–1600 (2005).
88. Nozaki, M. *et al.* Drusen complement components C3a and C5a promote choroidal neovascularization. *Proc. Natl. Acad. Sci. USA* **103**, 2328–2333 (2006).

Samsung Bioepis Exhibit No. 1002
Page 1176

Erratum: Funding crunch forces stem cell company to abandon therapies

Monya Baker
Nat. Biotechnol. 25, 951–952, 2007; published online 3 September 2007

In the version of this article originally published, in the penultimate paragraph, the name of the Monash University scientist was misspelled; the correct spelling is Colin Pouton. In addition, the statement in paragraph 2 "the SSCC, which is being supported by the same group that funded ESI, the Agency for Science, Technology and Research (A*STAR)" is incorrect. ESI is not funded by A*STAR. Its major investor is Bio-One Capital, a venture capital fund controlled through the Singapore Government's Economic Development Board. The SSCC is funded by A*STAR.

Erratum: Mice with a human touch

Christopher Thomas Scott
Nat. Biotechnol. 25, 1075–1077, 2007; published online 6 October 2007

In the version of this article initially published, it was incorrectly stated on p. 1076, paragraph 2, line four from bottom, that "Aya Jakobovits, originally at Cell Genesys and later at Abgenix, shepherded the XenoMouse through its entire development." Five of the six XenoMouse strains developed at Abgenix were initiated after Jakobovits left in 1997.

Erratum: Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria

Russell P Rother, Scott A Rollins, Christopher F Mojcik, Robert A Brodsky & Leonard Bell
Nat. Biotechnol. 25, 1256–1264 (2007); published online 7 November 2007; corrected after print 7 December 2007

In the version of this article initially published, on p. 1258, paragraph 2, the incorrect serum concentrations to reach steady state and the incorrect accumulation ratio (R_{acc}) for eculizumab were given. The corrected sentence reads "Eculizumab serum concentrations appear to reach steady state after ~57 days. At steady state, the eculizumab accumulation ratio (R_{acc}) was calculated to be 1.075." In addition, in Figure 5, in the PDF version only, the word "assessment" was cut off the bottom of the box beginning "September 2006." The final sentence should read "BLA and MAA submitted to FDA and EMEA, respectively; receive accelerated assessment." And in Table 1, the heading "Units of packed RBCs transfused/patient/month" now appears in a separate row. The errors have been corrected in the HTML and PDF versions of the article.

Corrigendum: A diverse family of thermostable cytochrome P450s created by recombination of stabilizing fragments

Yougen Li, D Allan Drummond, Andrew M Sawayama, Christopher D Snow, Jesse D Bloom & Frances H Arnold
Nat. Biotechnol. 25, 1051–1056 (2007); published online 26 August 2007; corrected after print 7 December 2007

In the version of this article initially published, the sentence starting with "The most thermostable 450 P450 chimera" contains an extra "450." The correct sentence should start as follows "The most thermostable P450 chimera". The error has been corrected in the HTML and PDF versions of the article.

© 2007 Nature Publishing Group http://www.nature.com/naturebiotechnology

Samsung Bioepis Exhibit No. 1002
Page 1177

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: February 15, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/148,839 Confirmation No.: 3159

Filed: May 6, 2016 Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT IN RESPONSE TO NON-FINAL OFFICE ACTION UNDER 37 C.F.R. § 1.111

Dear Sir:

In response to the Office Action dated August 16, 2016 (Paper No. 20160809), please amend the above-identified U.S. patent application as follows:

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the listing of claims which begins on page 3 of this paper.

Remarks/Arguments begin on page 4 of this paper.

Electronic Patent Application Fee Transmittal

Application Number:	15148839
Filing Date:	06-May-2016
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Attorney Docket Number:	AXJ-114BUSCN2

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 3 months with $0 paid	1253	1	1400	1400
Miscellaneous:				
STATUTORY OR TERMINAL DISCLAIMER	1814	1	160	160
		Total in USD ($)		**1560**

Electronic Acknowledgement Receipt	
EFS ID:	28359755
Application Number:	15148839
International Application Number:	
Confirmation Number:	3159
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Customer Number:	133485
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Filer Authorized By:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN2
Receipt Date:	15-FEB-2017
Filing Date:	06-MAY-2016
Time Stamp:	13:34:54
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$1560
RAM confirmation Number	021517INTEFSW00010277120080
Deposit Account	120080
Authorized User	Jill Sloper
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.16 (National application filing, search, and examination fees) 37 CFR 1.17 (Patent application and reexamination processing fees)	

37 CFR 1.19 (Document supply fees)

37 CFR 1.20 (Post Issuance fees)

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Extension of Time	AXJ_114BUSCN2_EOT.pdf	78950 efd909bda104f3f0a73129560aadb8904941b093	no	1
Warnings:					
Information:					
2	Assignee showing of ownership per 37 CFR 3.73	AXJ_114BUSCN2_373.pdf	2009072 1a84626b770071e0636bfccca4f0d3f704574150	no	2
Warnings:					
Information:					
3	Terminal Disclaimer Filed	AXJ_114BUSCN2_TD.pdf	1523021 83d2a74945b72cedd0678e90b794aec14c14e04e	no	2
Warnings:					
Information:					
4	Application Data Sheet	AXJ_114BUSCN2_Corrected_ADS.pdf	63289 ca51fd68ad58b92e7b3b617cffa4ad1a264c62ff	no	3
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
5	Request for Corrected Filing Receipt	AXJ_114BUSCN2_Request_Corrected_FR.pdf	113746 4b6e9b94730c4103289964cf50e91a6041d5cf10	no	2
Warnings:					
Information:					
6	CRF Statement Paper and CRF are the same	AXJ_114BUSCN2_Statement_re_SL.pdf	127982 2d3ac2e9389270beea35a314555b73d70ceb377c	no	2
Warnings:					

Information:					
7	Sequence Listing (Text File)	AXJ_114BUSCN2_Seq_Listing.txt	10244 	no	-
Warnings:					
Information:					
8		AXJ_114BUSCN2_Response_and_Exhibit_A.pdf	17336615 516e18760d8873245ad0ea1cf07ca239bb89d449	yes	18

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Applicant Arguments/Remarks Made in an Amendment	4	18
	Claims	3	3
	Specification	2	2
	Amendment/Req. Reconsideration-After Non-Final Reject	1	1

Warnings:					
Information:					
9	Fee Worksheet (SB06)	fee-info.pdf	32607 07c97410ae6a56ac9aea3c6a1c98c639dd3d0253	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	21295526		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/AIA/22 (03-13)
Approved for use through 7/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)

Docket Number (Optional): AXJ-114BUSCN2

Application Number	15/148,839-Conf. #3159	Filed	May 6, 2016

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Art Unit	1644	Examiner	J. L. Rogers

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

		Fee	Small Entity Fee	Micro Entity Fee	
☐	One month (37 CFR 1.17(a)(1))	$200	$100	$50	$
☐	Two months (37 CFR 1.17(a)(2))	$600	$300	$150	$
☒	Three months (37 CFR 1.17(a)(3))	$1,400	$700	$350	$ 1,400.00
☐	Four months (37 CFR 1.17(a)(4))	$2,200	$1,100	$550	$
☐	Five months (37 CFR 1.17(a)(5))	$3,000	$1,500	$750	$

☐ Applicant asserts small entity status. See 37 CFR 1.27.

☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

☐ A check in the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☒ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 12-0080 .

☒ Payment made via EFS-Web.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant.

☒ attorney or agent of record. Registration number 60,760 .

☐ attorney or agent acting under 37 CFR 1.34. Registration number _____ .

/Jill Gorny Sloper, Esq./
Signature

February 15, 2017
Date

Jill Gorny Sloper, Esq.
Typed or printed name

(617) 217-4630
Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

☐ * Total of 1 forms are submitted.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: February 15, 2017 Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: Alexion Pharmaceuticals, Inc.

Application No./Patent No.: 15/148,839 Filed/Issue Date: May 6, 2016

Titled: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Alexion Pharmaceuticals, Inc. (Name of Assignee) , a Corporation (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. [X] The assignee of the entire right, title, and interest.

2. [ ] An assignee of less than the entire right, title, and interest (check applicable box):

   [ ] The extent (by percentage) of its ownership interest is _____ %). Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

   [ ] There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. [ ] The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. [ ] The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

A. [ ] An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

B. [X] A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: Leonard Bell To: Alexion Pharmaceuticals, Inc.
   The document was recorded in the United States Patent and Trademark Office at Reel 038520, Frame 0957, or for which a copy thereof is attached.

2. From: Russell P. Rother To: Alexion Pharmaceuticals, Inc.
   The document was recorded in the United States Patent and Trademark Office at Reel 038520, Frame 0957, or for which a copy thereof is attached.

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

4. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

5. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

6. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☒ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

Signature	Date: 02/14/17
Todd Spalding	Vice President, Head of Intellectual Property
Printed or Typed Name	Title or Registration Number

==========
Sequence Listing was accepted.
If you need help call the Patent Electronic Business Center at (866) 217-9197 (toll free).
Reviewer: Kathleen Kalafus
Timestamp: [year=2017; month=2; day=16; hr=13; min=1; sec=58; ms=401;]
==========

Validated By CRFValidator v 1.0.5

Application No: 15148839 Version No: 2.0

Input Set:

Output Set:

Started: 2017-02-15 13:35:51.534
Finished: 2017-02-15 13:35:52.408
Elapsed: 0 hr(s) 0 min(s) 0 sec(s) 874 ms
Total Warnings: 10
Total Errors: 0
No. of SeqIDs Defined: 10
Actual SeqID Count: 10

Error code		Error Description
W	213	Artificial or Unknown found in <213> in SEQ ID (1)
W	213	Artificial or Unknown found in <213> in SEQ ID (2)
W	213	Artificial or Unknown found in <213> in SEQ ID (3)
W	213	Artificial or Unknown found in <213> in SEQ ID (4)
W	213	Artificial or Unknown found in <213> in SEQ ID (5)
W	213	Artificial or Unknown found in <213> in SEQ ID (6)
W	213	Artificial or Unknown found in <213> in SEQ ID (7)
W	213	Artificial or Unknown found in <213> in SEQ ID (8)
W	213	Artificial or Unknown found in <213> in SEQ ID (9)
W	213	Artificial or Unknown found in <213> in SEQ ID (10)

SEQUENCE LISTING

<110> ALEXION PHARMACEUTICALS, INC.

<120> TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS
BY AN INHIBITOR OF COMPLEMENT

<130> AXJ-114BUSCN2

<140> US 15/148,839
<141> 2016-05-06

<150> 13/426,973
<151> 2012-03-22

<150> 12/225,040
<151> 2009-05-13

<150> PCT/US2007/006606
<151> 2007-03-15

<160> 10

<170> PatentIn version 3.5

<210> 1
<211> 123
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 1

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ile Phe Ser Asn Tyr
            20                  25                  30


Trp Ile Gln Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45


Gly Glu Ile Leu Pro Gly Ser Gly Ser Thr Glu Tyr Thr Glu Asn Phe
    50                  55                  60


Lys Asp Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65                  70                  75                  80


Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
```

```
Ala Arg Tyr Phe Phe Gly Ser Ser Pro Asn Trp Tyr Phe Asp Val Trp
            100                 105                 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
        115                 120
```

<210> 2
<211> 448
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 2

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ile Phe Ser Asn Tyr
            20                  25                  30

Trp Ile Gln Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Glu Ile Leu Pro Gly Ser Gly Ser Thr Glu Tyr Thr Glu Asn Phe
    50                  55                  60

Lys Asp Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Tyr Phe Phe Gly Ser Ser Pro Asn Trp Tyr Phe Asp Val Trp
            100                 105                 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
        115                 120                 125

Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr
    130                 135                 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
```

```
145                 150                 155                 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
                165                 170                 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
            180                 185                 190

Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp
        195                 200                 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys
    210                 215                 220

Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser
225                 230                 235                 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                245                 250                 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro
            260                 265                 270

Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
        275                 280                 285

Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val
    290                 295                 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305                 310                 315                 320

Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr
                325                 330                 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
            340                 345                 350

Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
        355                 360                 365

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
    370                 375                 380
```

```
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
385                 390                 395                 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser
                405                 410                 415

Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
            420                 425                 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
        435                 440                 445
```

<210> 3
<211> 131
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 3

```
Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1               5                   10                  15

Leu Arg Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
            20                  25                  30

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Gly Ala Ser
        35                  40                  45

Glu Asn Ile Tyr Gly Ala Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys
    50                  55                  60

Ala Pro Lys Leu Leu Ile Tyr Gly Ala Thr Asn Leu Ala Asp Gly Val
65                  70                  75                  80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
                85                  90                  95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Asn
            100                 105                 110

Val Leu Asn Thr Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
```

```
        115                 120                 125

Lys Arg Thr
    130
```

<210> 4
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 4

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Gly Ala Ser Glu Asn Ile Tyr Gly Ala
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Gly Ala Thr Asn Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Asn Val Leu Asn Thr Pro Leu
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160
```

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 5
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 5
Asn Tyr Trp Ile Gln
1 5

<210> 6
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 6
Glu Ile Leu Pro Gly Ser Gly Ser Thr Glu Tyr Thr Glu Asn Phe Lys
1 5 10 15

Asp

<210> 7
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 7
Tyr Phe Phe Gly Ser Ser Pro Asn Trp Tyr Phe Asp Val
1   5   10

<210> 8
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      peptide

<400> 8
Gly Ala Ser Glu Asn Ile Tyr Gly Ala Leu Asn
1   5   10

<210> 9
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      peptide

<400> 9
Gly Ala Thr Asn Leu Ala Asp
1   5

<210> 10
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      peptide

<400> 10
Gln Asn Val Leu Asn Thr Pro Leu Thr
1   5

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/148,839	05/06/2016	1644	1740	AXJ-114BUSCN2	1	1

CONFIRMATION NO. 3159

CORRECTED FILING RECEIPT

133485
Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

OC000000089326078

Date Mailed: 02/21/2017

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Leonard BELL, Woodbridge, CT;
Russell P. ROTHER, Oklahoma City, OK;

Applicant(s)

Alexion Pharmaceuticals, Inc., New Haven, CT;

Power of Attorney: The patent practitioners associated with Customer Number 133485

Domestic Priority data as claimed by applicant

This application is a CON of 13/426,973 03/22/2012 ABN
which is a CON of 12/225,040 05/13/2009 ABN
which is a 371 of PCT/US2007/006606 03/15/2007

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 05/16/2016

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/148,839**

Projected Publication Date: Not Applicable

Non-Publication Request: No

Early Publication Request: No

Title

TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Preliminary Class

424

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

To: chris.schlauch@nelsonmullins.com,ipqualityassuranceboston@nelsonmullins.com,ipboston.docketing@nel
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 133485

Feb 22, 2017 03:24:49 AM

Dear PAIR Customer:

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 133485 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:
The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
15148839	APP.FILE.REC	02/21/2017	AXJ-114BUSCN2

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

Application Number	Application/Control No.	Applicant(s)/Patent under Reexamination	
	15/148,839	BELL ET AL.	

Document Code - DISQ	Internal Document – DO NOT MAIL

TERMINAL DISCLAIMER	☒ APPROVED	☐ DISAPPROVED
Date Filed : 15 February, 2017	**This patent is subject to a Terminal Disclaimer**	

Approved/Disapproved by:

PAMELA YOUNG

Technology Center: PLRC

Telephone: _____

U.S. Patent and Trademark Office

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: March 8, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/148,839 | Confirmation No.: 3159

Filed: May 6, 2016 | Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT | Examiner: J. L. Rogers

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST TO CORRECT INVENTORSHIP UNDER 37 C.F.R. § 1.48(a)

Dear Sir:

This request under 37 CFR § 1.48(a) is to add the following inventor to the above-referenced patent application:

Mark J. EVANS

This inventor was erroneously omitted from the above-referenced patent application at the time of filing.

Enclosed is a Corrected Application Data Sheet reflecting the names of all inventors in this application. A Request for Corrected Filing Receipt is also being submitted with this Request, along with the processing fee of $140.00 as set forth in 37 C.F.R. § 1.17(i); and the fee of $600.00 as set forth in 37 C.F.R. § 1.17(d). Please charge any additional fee to our Deposit Account No. 12-0080, under our Order No. AXJ-114BUSCN2.

Dated: March 8, 2017

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 217-4699 (Fax)
Attorney/Agent For Applicant

PTO/AIA/01 (06-12)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

As the below named inventor, I hereby declare that:

This declaration is directed to:
- ☐ The attached application, or
- ☒ United States application or PCT international application number 15/148,839 filed on 05/06/2016.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Mark J. EVANS Date (Optional): 6 March 2017

Signature: Mark J. Evans

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: March 8, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/148,839 | Confirmation No.: 3159

Filed: May 6, 2016 | Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT | Examiner: J. L. Rogers

Office of Initial Patent Examination's Filing Receipt Corrections
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CORRECTED FILING RECEIPT

Dear Sir:

Applicant hereby requests that a corrected Filing Receipt be issued in the above-identified patent application to reflect an update to the Inventor information.

Please correct the Inventor(s) information from:

Leonard BELL, Woodbridge, CT;
Russell P. ROTHER, Oklahoma City, OK;

to:

Leonard BELL, Woodbridge, CT;
Russell P. ROTHER, Oklahoma City, OK;
Mark J. EVANS, Radnor, PA;

Applicant additionally requests that all pertinent U.S. Patent and Trademark Office records relating to the subject application be changed to reflect this correction.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN2.

Dated: March 8, 2017

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 217-4699 (Fax)
Attorney/Agent For Applicant

Corrected Application Data Sheet

Inventor Information

Inventor Number::	1
Given Name::	Leonard
Family Name::	BELL
City of Residence::	Woodbridge
State or Province of Residence::	CT
Country of Residence::	US
Street of mailing address::	59 Tumblebrook Road
City of mailing address::	Woodbridge
State or Province of mailing address::	CT
Country of mailing address::	US
Postal or Zip Code of mailing address::	06525

Inventor Number::	2
Given Name::	Russell
Middle Name::	P.
Family Name::	ROTHER
City of Residence::	Oklahoma City
State or Province of Residence::	OK
Country of Residence::	US
Street of mailing address::	10325 Forest Meadow Drive
City of mailing address::	Oklahoma City
State or Province of mailing address::	OK
Country of mailing address::	US

Postal or Zip Code of mailing address::	73151
Inventor Number::	3
Given Name::	Mark
Middle Name::	J.
Family Name::	EVANS
City of Residence::	Radnor
State or Province of Residence::	PA
Country of Residence::	US
Street of mailing address::	617 Twinbridge Drive
City of mailing address::	Radnor
State or Province of mailing address::	PA
Country of mailing address::	US
Postal or Zip Code of mailing address::	19087

Application Information

Application Number::	15/148,839
Filing Date::	05/06/16
Title::	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
Attorney Docket Number::	AXJ-114BUSCN2

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Jill Gorny Sloper, Esq./	Date (YYYY-MM-DD)	2017-03-08
Name	Jill Gorny Sloper, Esq.	Registration Number	60,760

Electronic Patent Application Fee Transmittal

Application Number:	15148839
Filing Date:	06-May-2016
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Filer:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN2

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
LATE FILING FEE FOR OATH OR DECLARATION	1051	1	140	140
PROCESSING FEE, EXCEPT PROV. APPLS.	1830	1	140	140
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
CORRECTION OF INVENTORSHIP ON MERITS	1819	1	600	600
		Total in USD ($)		**880**

Electronic Acknowledgement Receipt	
EFS ID:	28562458
Application Number:	15148839
International Application Number:	
Confirmation Number:	3159
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Customer Number:	133485
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Filer Authorized By:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN2
Receipt Date:	08-MAR-2017
Filing Date:	06-MAY-2016
Time Stamp:	14:01:07
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$880
RAM confirmation Number	030917INTEFSW00000271120080
Deposit Account	120080
Authorized User	Jill Sloper
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.16 (National application filing, search, and examination fees) 37 CFR 1.17 (Patent application and reexamination processing fees)	

37 CFR 1.19 (Document supply fees)

37 CFR 1.20 (Post Issuance fees)

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request under Rule 48 correcting inventorship	Request_Under_148a.pdf	110240 7be8dd1ccf15397106f55de26de022de1f3f2c8	no	2
Warnings:					
Information:					
2	Oath or Declaration filed	Declaration_Evans.pdf	1269762 b6b6a96ff79935d4d960a1ca54ef4f0acd09afc8	no	1
Warnings:					
Information:					
3	Request for Corrected Filing Receipt	Request_Corrected_OFR.pdf	111680 2659fd3df46c0a751c001f06eb56abd40cf55e05	no	2
Warnings:					
Information:					
4	Application Data Sheet	Corrected_ADS_final.pdf	63465 cb23d4a1244098fa42176e69dfd824b79a92ae5e	no	3
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
5	Fee Worksheet (SB06)	fee-info.pdf	33625 15ebb0a20a30846e5cda1d471e32fd1de6c83027	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1588772		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/148,839	05/06/2016	Leonard BELL	AXJ-114BUSCN2

CONFIRMATION NO. 3159

133485
Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

37 CFR 1.48 ACKNOWLEDGEMENT LETTER

OC00000089797187

Date Mailed: 03/10/2017

NOTICE OF ACCEPTANCE OF REQUEST UNDER 37 CFR 1.48(a)

This is in response to the applicant's request under 37 CFR 1.48(a) submitted on 03/08/2017.

The request under 37 CFR 1.48(a) to correct the inventorship, to correct or update the name of an inventor, or to correct the order of names of joint inventors is accepted.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/ttran/

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/148,839	05/06/2016	1644	1880	AXJ-114BUSCN2	1	1

CONFIRMATION NO. 3159

UPDATED FILING RECEIPT

133485
Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

OC000000089797186

Date Mailed: 03/10/2017

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Leonard BELL, Woodbridge, CT;
Russell P. ROTHER, Oklahoma City, OK;
Mark J. EVANS, Radnor, PA;

Applicant(s)

Alexion Pharmaceuticals, Inc., New Haven, CT;

Power of Attorney: The patent practitioners associated with Customer Number 133485

Domestic Priority data as claimed by applicant

This application is a CON of 13/426,973 03/22/2012 ABN
which is a CON of 12/225,040 05/13/2009 ABN
which is a 371 of PCT/US2007/006606 03/15/2007

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted:

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/148,839**

Projected Publication Date: Not Applicable

Non-Publication Request: No

Early Publication Request: No

Title

TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Preliminary Class

424

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

To: chris.schlauch@nelsonmullins.com,ipqualityassuranceboston@nelsonmullins.com,ipboston.docketing@nel
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 133485

Mar 10, 2017 04:17:35 AM

Dear PAIR Customer:

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 133485 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:
The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
15148839	R48.REQ.G	03/10/2017	AXJ-114BUSCN2
	APP.FILE.REC	03/10/2017	AXJ-114BUSCN2

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/148,839	05/06/2016	Leonard BELL	AXJ-114BUSCN2	3159

133485 7590 04/04/2017

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
04/04/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

Office Action Summary	Application No. 15/148,839	Applicant(s) BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *2/15/2017*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-3* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-3* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

1. Claims 1-3 are pending and under consideration in the instant examination and read on a compositions which is a 300 mg eculizumab single-use dosage form comprising 30 ml of a 10 mg eculizumab/ml sterile, preservative free solution.

2. Applicant's two IDSs submitted on 2/15/2017 are acknowledged and have been considered.

Priority

3. The examiner acknowledges applicant's corrected priority claim to 3/15/2007. A Corrected Filing Receipt was mailed from the office on 2/21/2017, Reflecting that the earliest priority date for which priority is claimed is 3/15/2007, the filing date of PCT /US2007 /006606.

Claim Rejections - 35 USC§ 102

4. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless -
(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

5. Claim 2 is rejected under 35 U.S.C. 102(b) as being anticipated by Hillmen et al. (N Engl J Med 2004; 350: 552-9, IDS Ref. of 10/21/2016), as is evidenced by applicant's specification as filed at p.44 and Thomas et al. (Molecular Immunology, 33(17/18), pp.1389-1401, 1996).

Hillmen teaches a method for treating a human patient that suffers from paroxysmal nocturnal hemoglobinuria (PNH) by administering to the patient an effective amount of eculizumab (p. 552, "methods") which specifically binds to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b (p. 553, 3rd paragraph).

While Hillmen does not specifically recite that the antibody that binds CS comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID N0:4, the specification as filed at p. 44 discloses that SEQ ID NO: 2 and SEQ ID NO: 4 constitute the H and L chains for "eculizumab" and Hillmen, as noted supra, teaches administering "eculizumab". Hillmen further teaches that "eculizumab" is a recombinant humanized antibody that binds to C5 (p. 553, 3rd ~) and cites Thomas which in turn describes in detail humanization of the clone referred to as "5G1 .1" arrive at the humanized h5G1 .1 which retain both the affinity and blocking functions of the murine 5g1 .1 antibody (abstract). Accordingly and in absence of evidence to the contrary, the currently recited SEQ ID NO: 2 and SEQ ID NO: 4 which constitute the Land H chains of "eculizumab" are considered to be

inherent properties of the "eculizumab" anti-C5 humanized monoclonal antibody taught by Hillmen.

The reference teachings anticipate the claimed invention.

To the extent that applicant will argue that Hillmen names eculizumab but does not teach the precise sequences being currently claimed, please note the following:

35 USC 102(b) states:

A person shall be entitled to a patent unless -

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States,

While it is true that if the antibody was unknown prior, then a reference which names it does not enable that antibody. See MPEP 2121.02 sections I and II. See also 2121.01, "the stated test is whether a reference contains an 'enabling disclosure' " In re Hoeksema, 399 F.2d 269, 158 USPQ 596 (CCPA 1968). The disclosure in an asserted anticipating reference must provide an enabling disclosure of the desired subject matter; mere naming or description of the subject matter is insufficient, if it cannot be produced without undue experimentation." And ... "Such possession is effected if one of ordinary skill in the art could have combined the publication's description of the invention with his [or her] own knowledge to make the claimed invention." In re Donohue, 766 F.2d 531, 226 USPQ 619 (Fed. Cir. 1985)."

In the instant case, applicant's position is that the mere naming is insufficient. However, the extensive number of both patent literature and NPL documents as well as clinical trials indicates that eculizumab was widespread and well-known. As evidence of the availability and use of eculizumab the examiner refers applicant to the following documents, some of which are part of the Request for additional Information which is also part of this OA:

A) Exhibit A: from clinicalTrials.govClinicaltrials.gov (studies 41-60).

B) Exhibit B: (printout for NCT00005571), in particular page 3 indicating that the trial was ongoing in Bethesda MD, and bottom of page 1 indicating that the drug used was eculizumab.

C) Exhibit C: Clinicaltrials.gov from 6/23/2005 for NCT00098280

D) Exhibit D: Kaplan 2002. Current Opinion in Investigational Drugs 3(7): 10107-1023

E) Exhibit E: Appel et al. Kidney International 2006 70:S45-S50, in particular the paragraph spanning pp. S48-S49.

F) Wang (US 2005/0271660). The reference is a US Patent Application Publication claiming methods of using eculizumab (see claim 7), and is presumed to be enabled.

G) Rother (WO 2005/110481). See for example claims 10, 44-45, 79-80, 123, 125, 130 as well as page 17 line 26 - p. 18 line 12 (noting that the antibody from US Patent 6355245 is now named eculizumab).

H) Rother (US 2009/0028850). This is the publication of the national-stage entry of the '481 application above. Note that the underlying application (11/596382) contains claims to methods of using eculizumab; the office has considered these claims to be enabled. Note that the case recently was decided by the PTAB, and the Board agreed that claims drawn to administering eculizumab were obvious (and thus presumably enabled and operative) as of the filing date of that underlying application, 5/16/2005.

In re Antor Media Corp (CAFC, 2012) states that subject matter disclosed in a reference, whether it is a patent claim, other parts of a patent, or non-patent literature, is presumed to be enabling absent evidence to the contrary. Accordingly, it is the examiner's position that eculizumab was effectively in the public domain.

6. Claim 2 is rejected under 35 U.S.C. 102(b) as being anticipated by **Appel et al.** (Kidney International 70, S45-S50, 2006).

Appel teaches that eculizumab is a humanized monoclonal antibody that prevents the activation of complement component C5. Appel further teaches a randomized trial in 200 patients with eculizumab (S48 and spanning to S49)).

While Appel does not specifically recite that the antibody that binds CS comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID N0:4, in absence of evidence to the contrary, the currently recited SEQ ID NO: 2 and SEQ ID NO: 4 which constitute the Land H chains of "eculizumab" are considered to be inherent properties of the "eculizumab" anti-C5 humanized monoclonal antibody taught by Appel.

The reference teachings anticipate the claimed invention.

7. Claim 2 is rejected under 35 U.S.C. 102(b) as being anticipated by **Wang** (US2005/0271660).

Wang teaches an eculizumab that inhibits activation of the complement system (¶s11, 67, 171).

While Wang does not specifically recite that the antibody that binds C5 comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID N0:4, in absence of evidence to the contrary, the currently recited SEQ ID NO: 2 and SEQ ID NO: 4 which constitute the Land H chains of "eculizumab" are considered to be inherent properties of the "eculizumab" anti-C5 humanized monoclonal antibody taught by Wang.

The reference teachings anticipate the claimed invention.

Claim Rejections -35 USC§ 103

8. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained even though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

9. Claims 1-3 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hillmen et al. (N Engl J Med 2004; 350: 5S2-9), in view of Evans et al. (US 6,355,245 81, issued 3/12/2002), Wang (US 200S/0271660), as is evidenced by the parent specification of US Patent Application No. 13/426,973 at p. 16, lines 20-25.

Hillmen teaches a method for treating a human patient that suffers from paroxysmal nocturnal hemoglobinuria (PNH) by administering to the patient an effective amount of eculizumab (p. 552, "methods") which specifically binds to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b (p. 553, 3rd ¶). Hillmen teaches that patients received infusions of eculizumab weekly for four weeks, followed one week later by a 900 mg dose and then by a dose of 900 mg every other week through week 12 (p. 554 under "treatment schedule").

The prior art references differ from the claimed invention in the recitation that the composition is a 300 mg eculizumab single-use dosage form comprising 30 ml of a 10 mg eculizumab/ml sterile, preservative free solution as in claims 1 and 3.

However, Evans teaches antibody 5G1.1, which is the same as eculizumab (see parent specification US Patent Application No. 13/426,973, p. 16 lines 20-25), is effective at doses of 2.2, 3, or 5 mg per kg body weight; this corresponds to 154, 210, or 350 mg of antibody for a 70kg adult human ('245 patent, column 16 lines 10-30). Evans also teaches that eculizumab is therapeutically effective when as little as 210 mg are present (column 16 lines 10-15). Evans teaches that the antibodies are particularly advantageous in that they are highly active even when administered in low doses (col 8 lines 25-32), providing a

reasonable expectation of success in treating PHN with a dose less than what was disclosed by Hillmen. The reference teaches that it is within the skill of an artisan, particularly a physician, to determine the optimal dose (column 18 lines 1 5-30). Evans teaches formulations of the anti-C5 antibodies may be formulated in a pharmaceutically effective carrier such saline, which is considered to be a "preservative free solution". (col 18 lines 30-45).

However neither Hillmen nor '245 teaches a 300 mg single unit dosage as recited in the claims.

Nevertheless it would have been obvious to one of ordinary skill in the art to modify the teachings of Hillmen to administer 300 mg single unit dosages. Hillmen teaches that both 600 and 900 mg dosages can be used, and since Evans teaches that 210 or 350 mg of antibody would be expected to be therapeutically effective, arriving at the 300 mg dosage would be routine, convenient and obvious. One of ordinary skill in the art would have a reasonable expectation of success, as Evans teaches that the antibody exerts its effect at low doses.

Finally, **Wang** teaches that eculizumab is highly soluble, including at up to 30 mg per ml (paragraph 0171, Figure 10). Wang further teaches that pharmaceutically acceptable carriers include pyrogen-free water may be used in formulation of the antibody (1[71) sterile, preservative free solution). Therefore the artisan of ordinary skill would have had a reasonable expectation of success in making a 10 mg / ml solution of the antibody sterile, preservative free solution.

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

Applicant's arguments filed on 2/15/2107 were fully considered but were not considered persuasive.

Applicant asserts that prior to the 3/15/2007 effective filing date of the present application, the complete structure of eculizumab was not disclosed in the prior art; nor was it available to the public. Applicant asserts that neither Hillmen, Wang nor Evans teach or suggest the currently claimed sequence.

This argument was not considered persuasive for the following reasons because eculizumab is considered to have been in public use or on sale in this country more than one year prior to applicant's invention for the reasons stated supra in the anticipation rejection. Note the preponderance of evidence indicates that the antibody was in public use. The public availability of eculizumab is accordingly considered sufficient to have placed one of skill in the art in possession of applicant's currently claimed sequences for eculizumab.

Request for Additional Information Under 37 CFR 1.105

10. Applicant and the assignee of this application are required under 37 CFR 1.105 to provide the following information that the examiner has determined is reasonably necessary to the examination of this application.

11. In response to this requirement, please provide answers to each of the following interrogatories eliciting factual information:

(a) Studies #41-60 in the attached Exhibit A provide prima facie evidence that the methods now claimed were in use in this country more than a year before the document Exhibit A, please answer the following questions:

(i) Which is any of these studies disclose the same antibody sequences for eculizumab encompassed by the claims of the current application (i.e., SEQ ID NO: 2 and SEQ ID: 4)?

(ii) Whether any of the researcher(s) at each of the study sites identified in (i) above was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (ii) above?

(iv) Were the researchers for the study(s) identified in (i) above acting solely at the direction of the present inventors?

(b) the study entitled "Safety and Effectiveness of h5G 1.1-mAb for Dermatomyositis" listed on the attached Exhibit B please answer the following questions:

(i) Does this study disclose the same antibody sequences for eculizumab encompassed by the claims of the current application (i.e., SEQ ID NO: 2 and SEQ ID: 4)?

(ii) Whether any of the researcher(s) at the study site was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (i) above?

(iv) Were the researchers acting solely at the direction of the present inventors?

(C) For the study entitled "View of NCT00098280 on 2005_06_23" attached as exhibit C, please answer the following questions:

(i) Does this study disclose the same antibody sequences for eculizumab encompassed

by the claims of the current application (i.e., SEQ ID NO: 2 and SEQ ID: 4)?

(ii) Whether any of the researcher(s) the study sites was under any

confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (i) above?

(iv) Were the researchers acting solely at the direction of the present inventors?

(D) Exhibit D: "Eculizumab Alexion –Mariana Kaplan: please answer the following questions with respect to the study (445382) reference on p. 1018, 2nd ¶:

(i) Whether the trial used the same sequences for eculizumab as currently claimed (i.e., SEQ ID 2 and 4).

(ii) Whether any of the researcher(s) at the study site was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (i) above?

(iv) Were the researchers acting solely at the direction of the present inventors?

(E) Exhibit E (Appel "new approaches to the treatment of glomerular diseases), please answer the following questions with respect to the randomized, placebo-controlled trial in 200 patients referenced at the end of p. S48:

(i) Whether the trial used the same sequences for eculizumab as currently claimed (i.e., SEQ ID 2 and 4).

(ii) Whether any of the researcher(s) at study site was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (ii) above?

(iv) Were the researchers acting solely at the direction of the present inventors?

(F) With respect to the IDS submitted on 2/15/2017 which lists NPL literature, please answer the following questions:

(i) Do any of the studies referenced by the NPL documents disclose the same antibody sequences for eculizumab encompassed by the claims of the current application (i.e., SEQ ID NO: 2 and SEQ ID: 4)?

(ii) If the answer to (i) is in the affirmative, were any of the researcher(s) at each of the study sites was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (ii) above?

(iv) Were the researchers acting solely at the direction of the present inventors?

(G) On what date was eculizumab as encompassed by the current claims (i.e., SEQ ID Nos: 2 and 4) first offered for sale?

12. The applicant is reminded that the reply to this requirement must be made with candor and good faith under 37 CFR 1.56. Where the applicant does not have or cannot readily obtain an item of required information, a statement that the item is unknown or cannot be readily obtained may be accepted as a complete reply to the requirement for that item.

Double Patenting

13. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *Jn re Berg,* 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *Jn re Goodman,* 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi,* 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum,* 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel,* 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).
A timely filed terminal disclaimer in compliance with 37 CFR 1.321 (c) or 1.321 (d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(1)(1) - 706.02(1)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321 (b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/e TD-info-1.jsp.

14. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-19 of **US Patent No. 9,494,601,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '601 claim eculizumab (see claim 10 of the '601) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

15. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-18 of **US Patent No. 8,999,340,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '340 claim eculizumab (see claim 27 of the '340) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

16. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-17 of **US Patent No. 9,409,980,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '980 claim eculizumab (see claim 5 of the '980). The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

17. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 3-14 of **US Patent No. 9,556,263,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '263 claim eculizumab (see claims 2 and 4 of the '263) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml

anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

18. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-22 of **US Patent No. 9,352,035,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '035 claim eculizumab (see claim 1 of the '035) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

19. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-14 of **US Patent No. 9,415,102,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '102 claim eculizumab (see claim 15 of the '102) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

20. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-17 of **US Patent No. 9,409,980,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '980 claim eculizumab (see claim 3 of the '980). The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

21. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4 of **US Patent No. 9,447,176,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '176 claim eculizumab (see claim 1 of the

'176) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

22. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-5,7, 9-16 of US Patent No. 9,388,235, in view of Hillmen et al. (N Engl J Med 2004; 3SO: S52-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '23S claim eculizumab (see claim 31 of the '235) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

25. Claims 1-3 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 15-17, 58-59 of US Patent Application No: 11/127,438, in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '438 claim eculizumab (while not recited in the claims it is clearly a preferred embodiment; as the claim covers the product eculizumab this rejection is proper). The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-CS antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

25. Claims 1-3 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4 of **US Patent Application No: 15/243,386,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '386 claim eculizumab (see claim 1 of the '386) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5

antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

26. Claims 1-3 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4 of **US Patent Application No: 15/243,290,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '290 claim eculizumab (see claim 1 of the '290) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

27. Claims 1-3 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 94, 97-98 of **US Patent Application No: 15/282,464,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245 81, issued 3/12/2002) and Wang (US 2005/0271660).

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '464 claim eculizumab (see claim 94 of the '464) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

28. Claims 1-3 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 129 – 138 of **US Patent Application No: 15/148,491,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '491 claim eculizumab (see claim 129 of the '491) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5

antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

29. No claim is allowed.

30. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270- 5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel Kolker can be reached on (571) 272-3181. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

March 21, 2017

/JAMES ROGERS/

/DANIEL E. KOLKER/

Supervisory Patent Examiner, Art Unit 1644

Notice of References Cited	Application/Control No. 15/148,839	Applicant(s)/Patent Under Reexamination BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	Page 1 of 2

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-2005/0271660 A1	12-2005	Wang, Yi	C07K16/18	424/144.1
*	B	US-2009/0028850 A1	01-2009	Rother; Russell P.	C07K16/18	424/133.1
	C	US-				
	D	US-				
	E	US-				
	F	US-				
	G	US-				
	H	US-				
	I	US-				
	J	US-				
	K	US-				
	L	US-				
	M	US-				

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N	WO 2005110481 A2	11-2005	CANADA	ZHONG ZHEN	C07K16/18
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Hillmen et al. "Effect of Eculizumab on Hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria" NEJM, 350; 6, 2004, pp. 552-559
	V	Appel et al. "New approaches to the treatment of glomerular diseases" Kidney International (2006) 70, S45-S50
	W	69 Studies for eculizumab for clinical trials.gov, printed 2/28/2017, pp. 1-3
	X	Clinical Trials Identifier: NCT0098280, "Eculizumab to Treat Paroxysmal Nocturanl Hemoglobinuria" 6/23/2005, pp. 1-4

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Notice of References Cited	Application/Control No. 15/148,839	Applicant(s)/Patent Under Reexamination BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	Page 2 of 2

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
	A	US-				
	B	US-				
	C	US-				
	D	US-				
	E	US-				
	F	US-				
	G	US-				
	H	US-				
	I	US-				
	J	US-				
	K	US-				
	L	US-				
	M	US-				

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Safety and Effectiveness of h5G1.1-mAb for dermatomyositis" clinicaltrials.gov identifer NCT00005571, April 25, 2000, pp. 1-4
	V	Alexion Mariana Kaplan "Eculizumab" Current Opinion in Investigational Drugs 2002, 3(7): 10107-1023
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Eculizumab Alexion

Mariana Kaplan

Address
University of Michigan Medical Center
Division of Rheumatology
Int MED-Rheumatology
3918 TC
0358
Ann Arbor
MI 48109-1065
USA
Email: makaplan@umich.edu

Current Opinion in Investigational Drugs 2002 3(7):1017-1023
© PharmaPress ISSN 1472-4472

Eculizumab (5G1.1), a humanized monoclonal antibody that prevents the cleavage of human complement component C5 into its pro-inflammatory components, is under development by Alexion as a potential treatment for several chronic inflammatory diseases, including rheumatoid arthritis (RA) and nephritis [130673], [292328]. In January 2002, a phase IIb trial was initiated for RA [437814]. This trial was ongoing in April 2002, at which time, eculizumab was also in phase II trials for the treatment of membranous nephritis and lupus nephritis, and in earlier stage clinical trials for dermatomyositis and pemphigoid [446577]. The company is also developing a single-chain version of this antibody, pexelizumab, for use in acute cardiovascular indications [188760].

In October 2000, eculizumab was granted Orphan Drug status by the FDA for the treatment of dermatomyositis [385057]. In February 2002, the product received Orphan Drug designation for its use in patients with membranous nephritis [440583].

In September 2001, analysts at US Bancorp Piper Jaffray predicted eculizumab's launch for dermatomyositis and pemphigus in 2003, RA and nephritis in 2004, and chronic heart failure (CHF) after 2006 [426537]. In March 2002, analysts at US Bancorp Piper Jaffray predicted that the product would have peak worldwide sales in RA of US $175 million and US $400 million for nephritis. Sales for the RA indication are predicted to reach US $35 million in 2006, rising to US $110 million in 2008, and US $10 million in 2006, rising to US $50 million in 2008, in the US and the rest of the world, respectively. For the nephritis/other indication(s), sales are pegged at US $50 million in 2006, rising to US $200 million in 2008, and US $15 million in 2006, rising to US $100 million in 2008, again in the US and the rest of the world, respectively [446992].

Originator Alexion Pharmaceuticals Inc

Status Phase II Clinical

Indication Dermatological disease, Glomerulonephritis, Nephritis, Pemphigus, Psoriasis, Rheumatoid arthritis, Systemic lupus erythematosus

Action Complement cascade inhibitor

Biotechnology Monoclonal antibody, humanized

Synonyms 5G1.1, h5G1.1, C5 complement inhibitor (Alexion), h5G1.1scFv

CAS 5G1.1
Registry No: 339087-76-2

Introduction

The complement system comprises more than 30 human proteins in plasma and on cell surfaces that interact in a cascade sequence to mediate a variety of inflammatory events. These events include the opsonization of particles for phagocytosis, leukocyte activation and assembly of the membrane attack complex (MAC) [435653], [435654], [435718], [445228]. The system is responsible for killing and removing organisms, cells and cell components from the circulation which are recognized as foreign. In addition to host defense against bacterial agents, complement is involved in the disposal of immune complexes and the products of inflammatory injury, and serves as an interface between innate and adaptive immunity [435653].

Several complement proteins are cleaved during activation of the complement system and three pathways of activation have been described; the classical, the alternative and the lectin pathways [435653]. The pathways leading to the cleavage of C3 are triggered enzyme cascades. Downstream in these pathways, C3 is cleaved into C3a and C3b. C3a is released while C3b forms C5 convertases, which in turn cleave C5 to C5a and C5b. Following complement activation, pro-inflammatory peptides, such as the anaphylotoxins C3a and C5a, are generated and C5b-9 (MAC) is formed. Assembly of the MAC from the terminal components (C5 to C9) of the cascade leads to membrane damage. In addition, complement activation products, especially the anaphylotoxins, elicit a number of biological effects, including chemotaxis of leukocytes, degranulation of phagocytic cells, mast cells and basophils, smooth muscle contraction and the increase of vascular permeability [435653], [445228], [445736].

Under normal circumstances, the regulatory mechanisms of complement are balanced, so that the activation of complement occurs on the surface of invading microorganisms and the deposition of complement on normal cells and tissues is usually limited [435653], [445228]. If the mechanisms that regulate this balance are abnormal, the complement system might then cause tissue injury [435656]. Excessive or inappropriate activation of the complement system can lead to harmful and life-threatening consequences which are secondary to severe inflammatory tissue destruction. These consequences can manifest clinically in a variety of ways, including multiple organ failure, hyperacute graft rejection and septic shock [435656]. Inappropriate complement activation and its deposition on host cells can also lead to direct complement-mediated cell lysis of target structures, or indirectly to tissue destruction due to the generation of powerful mediators of inflammation [435653], [445228].

Complement plays an important role in the pathogenesis of many autoimmune and inflammatory diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) [435694], [445236] and dermatomyositis [445242]. It is also responsible for disease states associated

with bio-incompatibility, including transplant rejection [445249]. In addition, complement activation plays an important role in the pathogenesis of cardiac and intestinal ischemia/reperfusion injury [445251]. Other conditions in which complement activation has been proposed to play a role include stroke [445254], multiple sclerosis [445384], pancreatitis [445255], psoriasis [445272], Alzheimer's disease [445278] and prion-induced diseases [445286].

Therapeutic inhibition of complement is, therefore, potentially important in protecting against the development of certain diseases and it has been shown to be useful in animal models of sepsis, intestinal ischemia/reperfusion injury, myocardial reperfusion injury, nephritis, arthritis and graft rejection.

Several complement inhibitors are currently under investigation [212463], [225318], [281110], [435656], [435722], [435723], [435725], [436916]. Eculizumab (5G1.1), under development by Alexion Pharmaceuticals Inc, is a humanized C5 inhibitory monoclonal antibody (mAb) that prevents the cleavage of human complement component C5 into its pro-inflammatory components and targets the complement components, C5a and C5b-9 [445382]. As mentioned, the early steps of the complement cascade play an important role in the removal of infectious agents and several normal functions of the immune system. The rationale of specifically blocking C5 is that the normal upstream disease-preventing functions of complement remain intact, while the production of the abnormal downstream disease-causing actions of complement are blocked. This antibody, therefore, potentially avoids the disruption of antibacterial-protective mechanisms, which are believed to be mediated primarily through C3b [438165].

Eculizumab is undergoing phase II clinical trials for a variety of chronic inflammatory conditions, and is being developed as a potential treatment for RA, SLE and nephritis [190673], [292328], [412090]. The drug is currently in phase II trials for nephritis and has completed phase I/II trials in SLE and phase II trials in RA [328001], [335241], [335995], [396672]. Eculizumab is also currently undergoing phase I trials for psoriasis and pemphigus [384948], and has been granted Orphan Drug status by the FDA for the treatment of dermatomyositis [385057], and membranous nephritis [440583].

Alexion has also developed a short-acting C5 complement inhibitor that is a humanized single chain mAb fragment. This compound is known as pexelizumab (formerly 5G1.1-SC) and it is being tested for the treatment of acute cardiovascular diseases associated with complement activation, including acute myocardial infarction (MI), unstable angina and revascularization/reperfusion therapies. This drug was designed to have the advantage of enhanced tissue penetration, desirable for effective treatment of these acute life-threatening conditions [186760]. Pexelizumab and eculizumab are currently in at least eight clinical development programs [435963]. A phase III clinical study with pexelizumab in cardiopulmonary bypass patients has been initiated. In addition, two large phase II studies with pexelizumab in acute myocardial infarction (MI) patients are under way. Since pexelizumab has recently been evaluated, this review will focus primarily on eculizumab.

Synthesis and SAR

Humanized 5G1.1, Fab and scFv molecules were produced by grafting the complementary determining regions of 5G1.1 on to human framework regions [258884]. Competitive ELISA analysis indicated that no framework changes were required in the humanized variable regions for retention of high affinity binding to C5, even at framework positions predicted by computer modeling to influence CDR canonical structure. The humanized Fab and scFv molecules blocked complement-mediated lysis of chicken erythrocytes and porcine aortic endothelial cells in a dose-dependent fashion. Complete complement inhibition occurred at 3-fold molar excess, relative to the human C5 concentration. Humanized 5G1.1 scFv also effectively blocked C5a generation. In addition, intact humanized 5G1.1 antibody blocked human complement lytic activity at concentrations identical to the original murine monoclonal antibody, indicating that humanized 5G1.1 and its derivatives retained both the affinity and blocking functions of murine 5G1.1 antibody [258883], [258884]. 5G1.1 is supplied as a sterile, non-pyrogenic solution (2 mg/ml) for iv injection [212467], [258884] and it has picomolar affinity for a sequence within human C5. 5G1.1 can be administered either intramuscularly or subcutaneously, allowing self-administration [438165].

Pharmacology

Heart disease

There is a growing body of evidence pointing to a substantial disease-promoting role of complement components in MI and unstable angina [445289]. Activation of the complement cascade in MI patients is shown by elevated levels of activated complement byproducts in the blood of patients during acute MI and by the deposition of activated complement components in damaged heart tissue [445382]. Interestingly, terminal complement components play a key role in the apoptotic process in heart tissue during MI [241313], and blockade of the complement cascade at C5 has been shown to substantially reduce myocardial apoptosis and tissue damage in rats [241313]. Complement activation was also found in patients undergoing cardiopulmonary bypass (CBP) [445311]. This finding was associated with increased patient morbidity, including MI, stroke, cognitive dysfunction and blood loss [445311].

Studies in primates demonstrated that inhibition of complement activation reduces heart damage associated with coronary ischemia and reperfusion in preclinical models. Furthermore, treatment of hypoxic/reoxygenated human umbilical vein endothelial cells (HUVECs) with h5G1.1 scFv attenuated C5b-9 deposition, NFκB translocation and vascular cell adhesion molecule (VCAM)-1 expression [363137].

Intestinal and lung ischemia-reperfusion

A recent study reported that anti-C5a therapy significantly improved intestinal ischemia-reperfusion tissue injury as well as lung injury in rats [445313]. These results suggested that C5 inhibition could also limit the complaints in the broader general surgery population as well as in patients with ulcerative colitis [357827] Interestingly, C5 inhibition was associated with a significant reduction in tumor necrosis factor (TNF)α levels [357827].

Inflammatory arthritis

Animals deficient in the complement component C5 are not susceptible to the onset of active arthritis [363495]. In the absence of C5 cleavage, the presence of TNFα is not sufficient to promote arthritis. A recent study reported that administration of anti-C5 mAb ameliorates the established collagen-induced arthritis in mice and rats [190673], [212456]. Systemic administration of the anti-C5 mAb effectively inhibited terminal complement activation *in vivo* and prevented the onset of arthritis in immunized animals. In addition, anti-C5 mAb treatment was also highly effective in ameliorating established disease. These results suggested a critical role for activated terminal complement components, not only in the induction, but also in the progression of collagen-induced arthritis [190673], [212456].

Renal disease and lupus

A C5 inhibitor was administered in both an acute and rapid progressive glomerulonephritis (RPGN) model that normally leads to crescentic changes, the most common histological lesion observed in lupus nephritis. The inhibitor was also administered to the NZB/NZW F1 lupus-prone mouse model. C5 inhibition prevented histological and clinical development of nephritis in the RPGN model and C5 inhibitor administration in the lupus-prone mouse model reduced histologic and biochemical evidence of kidney disease. Survival at 10 months was markedly improved from 5% in placebo-treated lupus-prone mice to 85% in C5 inhibitor-treated lupus-prone mice [190673], [223608], [280073].

Hematological diseases

C5 inhibition has also been shown to prevent platelet loss in a model of idiopathic thrombocytopenic purpura (ITP) [392060].

Metabolism

5G1.1 blocks complement activity for 1 to 2 weeks after a single dose [438165].

Toxicity

No toxicity has been reported in humans [228105], [392382] or rhesus monkeys [212462]. Interestingly, a recent study reported that C5 plays an important role in liver regeneration, strongly implicating the complement system as a crucial regulatory component of hepatic homeostasis. C5-deficient mice show severely defective liver regeneration and persistent parenchymal necrosis after exposure to CC_{l_4}. In addition, these mice show marked delays in the re-entry of hepatocytes into the cell cycle (S phase) and diminished mitotic activity. Reconstitution of C5-deficient mice with murine C5 or C5a significantly restores hepatocyte regeneration after toxic injury. Furthermore, blockade of the C5a receptor abrogates the ability of hepatocytes to proliferate in response to liver injury [445317]. The role of eculizumab on hepatic regeneration is unclear at this point.

C5 is also known to mediate chemotactic and activation events that are the basis for granulomatous responses during murine tuberculosis. Mycobacterium tuberculosis-infected mice with natural deficiency in C5 are unable to develop productive granulomatous responses, and are impaired in limiting organism growth within the lung [445321].

Clinical Development

Phase I

Rheumatoid arthritis

A phase I/II, multicenter, double-blind, placebo-controlled, dose-escalating trial tested the role of eculizumab in ameliorating RA. The trial was designed to gather clinical data on the safety profile and biological effects of a single administration of eculizumab in RA. The preliminary analysis demonstrated that the drug was well tolerated and was associated with a significant reduction in disease activity in these patients [445382]. The safety and biological activity of the antibody was examined in 40 patients with mild-to-moderate RA, each of whom received a single dose of eculizumab (0.1 to 8 mg/kg). The drug was safe and well tolerated, and showed no detectable immunogenicity in the population studied. Furthermore, a single dose of eculizumab potently and rapidly blocked complement activity in a dose-dependent fashion for up to 2 weeks [353141]. Complement inhibition after a single dose was associated with a beneficial clinical effect, with the highest dose (8 mg/kg; n = 6) resulting in a significant 30% reduction in C-reactive protein levels, compared to placebo-treated patients in which C-reactive protein increased by 24%. Eculizumab was also associated with a trend in reductions in other measurement of disease activity, including tender joint count, swollen joint count, patients' global assessment of disease and patients' global assessment of pain [291142], [305950], [322159], [335241], [347482], [355995].

Systemic lupus erythematosus

Alexion has completed preliminary analysis of the phase I study of eculizumab in SLE. The trial was designed to gather data on pharmacodynamics, safety and biological effects of a single administration of the drug in SLE patients. Eculizumab was well tolerated and its administration was associated with significant reduction in the incidence of proteinuria. The phase I, double-blind, placebo-controlled, dose-escalating trial examined the safety and biological activity of eculizumab in 24 patients with mild SLE, each of whom received a single dose of the antibody (0.1 to 8 mg/kg). There was no detectable immunogenicity in the patients studied and a single dose of antibody blocked complement activity in patients for up to 2 weeks. Administration of a single dose of 8 mg/kg was associated with significant decrease in proteinuria [292328], [328001].

Dermatomyositis

In 2001, Alexion completed a 2-month, phase I, pilot safety trial of eculizumab in 13 dermatomyositis patients with persistent dermatomyositis, undergoing concomitant treatment with moderate doses of methotrexate or steroids. The patients were evaluated in either a placebo (n = 3) or a single drug treatment arm (n = 10). Drug treatment consisted of eculizumab (8 mg/kg/week iv) for 3 weeks and then 8 mg/kg every 2 weeks for up to 2 months. The patients were evaluated after 2 months of treatment for safety and for trends in clinical improvement. In this placebo-controlled, multicenter trial, the drug was safe and well tolerated and associated with an improvement in skin rash. Exploratory clinical measurements included clinical and laboratory assessments of skin rash and muscle strength. There were consistent trends in improvements with drug administration in subjective and objective

Samsung Bioepis Exhibit No. 1002
Page 1239

measurements of skin rash during the 2-month trial. While there was little baseline skin inflammation in the placebo group, a majority of drug-treated patients who completed the trial experienced an improvement of ≥ 50% in their skin rash score [354030], [434351], [435888].

Pemphigoid

Published observations have shown that clinical improvement of pemphigoid is associated with reduced levels of complement activation in the previously affected skin, further supporting the rationale for testing the C5 inhibitor in this disease. Phase I trials of eculizumab in pemphigoid syndrome are ongoing [361798], [426537], [435888], but no data are currently available.

Psoriasis

A single-center, double-blind, placebo-controlled study was designed to evaluate the safety profile and clinical effects of eculizumab in severe psoriasis [352110]. In June 2001, Alexion completed a phase I 'pilot' safety trial involving 40 psoriasis patients, which showed that the drug was well tolerated. Drug administration did not influence the clinical outcome as measured by Psoriasis Area and Severity Index (PASI) score, although favorable trends in certain measures of disease activity were observed. Drug administration dose-dependently blocked hemolytic activity in the blood of treated patients and dose-dependently reduced deposition of activated terminal complement in psoriatic plaques [412091].

Phase II

Rheumatoid arthritis

In a phase II trial in 209 patients, eculizumab administration appeared to be safe and well tolerated and the adverse event profile was comparable to placebo. Patients were treated with placebo, eculizumab (8 mg/kg iv) once per week for 4 weeks and then once every month (induction/monthly group); eculizumab (8 mg/kg iv) once per week for 4 weeks followed by once every 2 weeks (induction/biweekly group); or, eculizumab (8 mg/kg iv) once every 2 weeks (biweekly group). The results after 3 months of treatment showed that the induction/monthly group met the primary endpoint of the trial (improvement in American College of Rheumatology response criteria score (ACR) 20 after 3 months of treatment), while the induction/biweekly and biweekly groups did not statistically meet the endpoint. The ACR20 response in the induction/monthly group was 44% as compared to 18% ACR20 in the placebo group, by per protocol analysis. Both induction/monthly and induction/biweekly groups also met the secondary endpoint of changes in C-reactive protein after 3 months of therapy [429348].

In patients with elevated C5b-9 levels over 200 ng/ml, the ACR20 at 3 months of treatment were dose-dependent. The ACR20 results obtained in patients with elevated baseline C5b-9 levels were: placebo (9%); biweekly (33%); induction/monthly (57%); and induction/biweekly (50%). Further data from this trial are to be released following unblinding [397163], [429348].

Alexion has also started a phase IIb trial in RA patients. The trial is designed to assess the safety and efficacy of eculizumab and to confirm the most efficacious dose regimen. The trial will consist of approximately 300 patients with mild-to-moderate disease who are being treated concomitantly with disease-modifying antirheumatic drugs such as methotrexate or leflunomide. The trial will consist of three treatment arms; patients will be treated with placebo; eculizumab (8 mg/kg/week iv) for 4 weeks and then once every month; or eculizumab (8 mg/kg/week) for 4 weeks and then bimonthly. The patients will be evaluated after a 6-month drug phase for safety and efficacy, and the primary efficacy endpoint will be the ACR20 score [437814].

Systemic lupus erythematosus and nephritis

In August 1999, Alexion initiated a phase II study with eculizumab in lupus patients suffering from membranous nephritis [335995]. The trial is expected to enroll approximately 40 lupus nephritis patients at four clinical sites in the US and has been designed to test the safety and biological efficacy of chronic administration of eculizumab for up to 6 months [412090]. In September 2001, Alexion expected phase II results to be available in mid-2002 [426537].

Side Effects and Contraindications

In clinical trials to date, the drug was well tolerated and showed no detectable immunogenicity in the patients studied. No major adverse reactions have been reported. In the dermatomyositis trial, the most common side effects were headache and rash, and it appeared that these side effects were comparable to the placebo group. In the psoriasis trial, the most common side effects were headaches and unspecified pain. The most common side effects in the RA trial were comparable to placebo and included diarrhea and headaches [429348], [434351].

Patent Commentary

In March 2002, Alexion was issued US-06355245 entitled 'C5-specific antibodies for the treatment of inflammatory diseases.' The patent covers the composition of Alexion's lead drug candidates, eculizumab and pexelizumab, and other C5-binding anti-inflammatory antibodies [443717]. WO-09529697, entitled 'methods and compositions for the treatment of glomerulonephritis and other inflammatory diseases' describes a method for the possible treatment of glomerular inflammation and enlargement, involving the administration of low dosages of anti-C5 antibodies. WO-09609043 describing a method for the treatment of established joint inflammation is claimed in which a C5 blocker is administered.

Current Opinion

C5 inhibitors appear to intervene at a point that allows preservation of the anti-inflammatory and antibacterial responses at the C3 level, while conceivably inhibiting the downstream disease-causing actions. Selective suppression of this immune response could certainly provide a significant therapeutic advantage compared to existing therapies. Eculizumab blocks the production of harmful complement components and appears to be promising for the treatment of various inflammatory diseases, including RA, SLE and a variety of conditions with skin involvement, including dermatomyositis, pemphigoid and psoriasis. There are other conditions in which C5a seems to play an important role, including asthma and HIV infection, and these conditions might potentially become therapeutic targets in which C5a inhibition could be useful [445327], [445336].

Samsung Bioepis Exhibit No. 1002
Page 1240

Since complement activation has both positive and negative effects, the risk associated with complement inhibition is probably not negligible and long term studies will be necessary to assess possible risks. In particular, a theoretical concern is the role that C5 plays in hepatic regeneration and in combating granulomatous infections such as tuberculosis. Long term studies will determine whether C5 inhibition could have deleterious effects in these processes.

So far, limited animal and human studies have shown that eculizumab inhibits complement in a dose-dependent manner, is well tolerated and has no significant side effects. The results have been encouraging so far but more clinical trials are needed. There are still concerns that this drug does not block activation of the early stages of the complement system. This inhibitor allows the generation of C3a. While C3a is considered a less potent inflammatory molecule than C5a, the C3a receptor tissue expression appears to be much broader than originally expected [445349], [445352], and recent studies have shown that C3a can induce the production of inflammatory cytokines in a fashion similar to C5a [445354]. C3a could, in theory, play a significant role in inflammatory conditions and acute-phase response even after C5 inhibition. Nevertheless, if clinical trials prove successful, this drug as well as pexelizumab, could have a broad range of applications where complement-mediated inflammation contributes to disease pathology. There are currently no biological therapies on the market with the actions and specificity of these drugs and eculizumab could have a very strong therapeutic potential.

Development history

Developer	Country	Status	Indication	Date	Reference
Alexion Pharmaceuticals Inc	US	Phase II clinical	Rheumatoid arthritis	09-JUL-98	291142
Alexion Pharmaceuticals Inc	US	Phase II clinical	Nephritis	12-AUG-99	335995
Alexion Pharmaceuticals Inc	US	Phase I clinical	Psoriasis	06-OCT-00	384948
Alexion Pharmaceuticals Inc	US	Phase I clinical	Dermatological disease	06-OCT-00	384948
Alexion Pharmaceuticals Inc	US	Phase I clinical	Pemphigus	06-OCT-00	384948
Alexion Pharmaceuticals Inc	US	Preclinical	Glomerulonephritis	28-JUL-97	190673
Alexion Pharmaceuticals Inc	US	NDR	Systemic lupus erythematosus	11-APR-02	-

Literature classifications

Biology

Study Type	Effect Studied	Experimental Model	Result	Reference
In vivo	Anti-arthritic effect.	Collagen-induced arthritis in mice and rats.	An anti-C5 antibody both prevented the onset of disease and reduced established disease.	190673 212456
In vivo	Reduction in evidence of kidney disease.	NZB/NZW F1 lupus-prone mouse model.	An anti-C5 antibody reduced both the histological and biochemical signs of nephritis, and increased survival to 85% at 10 months.	232606

Metabolism

Effect Studied	Model Used	Result	Reference
Pharmacokinetics.	Complement activity.	A single dose of eculizumab blocks complement activity for 1 to 2 weeks.	436165

Clinical

Effect Studied	Model Used	Result	Reference
Safety and efficacy.	Phase I trial in 40 psoriasis patients.	Eculizumab was well tolerated and dose-dependently reduced complement activity in patients. Favorable trends were observed in PASI, although these were not significant.	412091
Safety and efficacy.	Phase I trial in 13 patients with persistent dermatomyositis.	Eculizumab (6 mg/kg/week iv) for 5 weeks, then every 2 weeks for up to 2 months, was safe and well tolerated. Drug treatment reduced skin rash score by ≥ 50%.	434351
Safety and efficacy.	Double-blind, placebo-controlled, phase I, dose-escalating trial in 24 patients with mild SLE.	Eculizumab (0.1 to 6 mg/kg) was safe and well tolerated, and no detectable immunogenicity was observed. The highest dose significantly reduced proteinuria.	328001
Safety and efficacy.	Phase II trial in 209 RA patients.	Eculizumab produced ACR20 in 44% of the patients treated. Adverse effects were similar to placebo, with nausea and diarrhea being the most common complaints.	429348
Safety and efficacy.	Phase II trial in RA patients in whom the C5b-9 levels were > 200 ng/ml.	A total of 57% of patients achieved ACR20 when treated with induction/monthly treatment of eculizumab.	429348

Associated patent

Title Method for reducing immune and hemostatic dysfunctions during extracorporeal circulation.

Assignee Alexion Pharm Inc

Publication WO-09526540 28-SEP-95

Priority US-00217391 23-MAR-94

Inventors Rollins SA, Smith BR, Squinto SP.

Associated references

160760 **Anti-complement MAbs in arthritis.** *SCRIP* 1995 2065 26

190673 **Alexion complement inhibitor shows efficacy in animal models of lupus nephritis: results presented at the American College of Rheumatology.** Alexion Pharmaceuticals *PRESS RELEASE* 1995 October 25
• *This press release documents the theoretical actions of eculizumab and its effectiveness in reducing histologic and clinical development of nephritis in a murine model of lupus nephritis.*

212456 **Anti-C5 monoclonal antibody therapy prevents collagen-induced arthritis and ameliorates established disease.** Wang Y, Rollins SA, Madri JA, Matis LA *PROC NATL ACAD SCI USA* 1995 **92** 19 8955-8959

212462 ***In vitro* and *in vivo* inhibition of complement activity by a single-chain Fv fragment recognizing human C5.** Evans MJ, Hartman SL, Wolff DW, Rother RP, Korin AJ, Thernlen DM, Grijalva GA, Mueller JP, Nye SH, Squinto SP *et al MOL IMMUNOL* 1995 **32** 16 1183-1195
• *This paper documents the effectiveness of the anti-C5 monoclonal antibody (N19-8 scFv) at inhibiting complement-dependent myocardial injury in isolated mouse heart. In other studies, the ability of the antibody to inhibit complement hemolytic activity in rhesus monkeys while being well tolerated at doses up to 100 mg was documented.*

212463 **Complement-specific antibodies: designing novel anti-inflammatories.** Matis LA, Rollins SA *NAT MED* 1995 **1** 8 838-842

212467 **Rapid expression of an anti-human C5 chimeric Fab utilizing a vector that replicates in COS and 293 cells.** Evans MJ, Hartman SL, Wolff DW, Rollins SA, Squinto SP *J IMMUNOL METHODS* 1995 **184** 1 123-138
• *This paper describes the generation and expression of the 5G1.1-SC antibody.*

225318 **Anti-C5 monoclonal antibody: a novel anti-inflammatory agent.** *EXP OPIN THER PAT* 1996 **6** 11 1229-1230

228105 **Alexion announces positive phase I results with C5 inhibitor.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 1996 December 11
• *This press release reports on the ability of 5G1.1-SC to block complement activation after intravenous administration into healthy male volunteers.*

232006 **Amelioration of lupus-like autoimmune disease in NZB/WF1 mice after treatment with a blocking monoclonal antibody specific for complement component C5.** Wang Y, Hu Q, Madri JA, Rollins SA, Chodera A, Matis LA *PROC NATL ACAD SCI USA* 1996 **93** 16 8563-8568
• *This paper documents the effectiveness of an intact anti-C5 antibody in reducing glomerulonephritis and increasing survival in a murine model of systemic lupus using autoimmune mice.*

241613 **New cell suicide pathway discovered: Alexion C5 inhibitor reported to block apoptosis during myocardial infarction.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 1997 April 04

250883 **Safety, pharmacokinetics, and immunogenicity of intravenous administration of h5G1.1-scFv in humans.** Fitch JCK, Eleftetiades JA, Matis LA, Evans MJ, Rinder HM, Rollins SA, Allred SL, Hines RL *J AM COLL CARDIOL* 1997 **29** 2 Suppl A 344A

258884 **Inhibition of complement activity by humanized anti-C5 antibody and single-chain Fv.** Thomas TC, Rollins SA, Rother RP, Giannoni MA, Hartman SL, Elliott EA, Nye SH, Matis LA, Squinto SP, Evans MJ *MOL IMMUNOL* 1996 **33** 17-18 1389-1401

280073 **Alexion Pharmaceuticals Inc.** *ANALYST REPORT* 1997 September 24

281110 **Novel complement inhibitors.** Lezewski MK, Atkinson JP *EXPERT OPIN INVESTIG DRUGS* 1998 **7** 3 323-332

291142 **Alexion begins C5 inhibitor clinical trial for rheumatoid arthritis - alexion moves into clinic treating autoimmune disorder with second anti-inflammatory drug candidate.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 1998 July 07

292308 **Alexion begins C5 inhibitor phase I/II clinical trial for lupus - Alexion treating second autoimmune disorder with anti-inflammatory drug candidate 5G1.1.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 1998 July 23

305950 **Drug development pipeline.** *DRUG DEV PIPELINE* 1998 **3** 7 1

322150 **Alexion announces positive clinical results from phase I/II study of C5 complement inhibitor in rheumatoid arthritis patients.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 1999 April 21

328001 **Alexion reports positive results from phase I clinical trial of C5 inhibitor for systemic lupus.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 1999 June 15

335241 **Alexion initiates phase II efficacy study of C5 complement inhibitor in rheumatoid arthritis patients.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 1999 August 04

335995 **Alexion initiates phase II efficacy study of C5 complement inhibitor in kidney disease patients.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 1999 August 11

347462 **Alexion's 5G1.1 drug reduces clinical signs and symptoms of rheumatoid arthritis in completed trial - results presented at 63rd Annual Meeting of the American College of Rheumatology.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 1999 November 15

352110 **Alexion starts clinical development with C5 inhibitor 5G1.1 in psoriasis patients.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2000 January 11

353137 **Endothelial nuclear factor-kappaB translocation and vascular cell adhesion molecule-1 induction by complement: inhibition with anti-human C5 therapy or cGMP analogues.** Collard CD, Agah A, Reenstra W, Buras J, Stahl GL *ARTERIOSCLER THROMB VASC BIOL* 1999 **19** 11 2623-2629

353141 **A single dose, placebo controlled, double blind, phase I study of the humanized anti-C5 antibody h5G1.1 in patients with rheumatoid arthritis.** Jain RI, Moreland LW, Caldwell JR, Rollins SA, Mojcik CF *ARTHRITIS RHEUM* 1999 **42** 9 Suppl S77

354030 **Alexion starts clinical development with C5 inhibitor 5G1.1 in dermatomyositis patients - C5 inhibitor targets inflammatory muscle disorder.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2000 February 01

357827 **Alexion's anti-inflammatory C5 inhibitor prevents organ damage associated with GI ischemia - Preclinical results extend potential efficacy of C5 inhibitor therapy to treatment of complications of general surgical procedures and ulcerative colitis.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2000 March 01

361798 **Alexion starts clinical development with C5 inhibitor 5G1.1 in pemphigoid patients - C5 inhibitor targets inflammatory skin disorder.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2000 April 06

363466 **Alexion and Yale Scientists demonstrate that C5 is required for active arthritis.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2000 April 18

364948 **Alexion Pharmaceuticals reports fourth quarter and year end results.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2000 October 05

365057 **Alexion receives orphan drug status for C5 inhibitor antibody 5G1.1 in dermatomyositis patients - Regulatory status provides for market exclusivity.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2000 October 06

382090 **Alexion's anti-inflammatory C5 inhibitor prevents chronic autoimmune platelet disease - preclinical results presented at American Society of Hematology.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2000 December 04

382982 **Pharmacology and biological efficacy of a recombinant, humanized, single-chain antibody C5 complement inhibitor in patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass.** Fitch JCK, Rollins S, Matis L, Alford B, Aranki S, Collard CD, Dewar M, Elefteriades J, Hines R, Kopf G, Kraker P *et al CIRCULATION* 1999 **100** 26 2499-2506

396672 **Alexion reports initial analysis of clinical safety and efficacy data from phase IIb cardiopulmonary bypass trial: pexelizumab significantly reduced composite of death or myocardial infarction in CABG patients.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2001 January 23

397163 **Alexion reports interim analysis of clinical safety and efficacy data from phase II rheumatoid arthritis trial.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2001 January 29

412090 **Alexion commences phase II lupus nephritis clinical trial.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2001 June 07

412091 **Alexion announces completion of phase I psoriasis pilot safety study.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2001 June 07

426507 **Equity research notes: Alexion Pharmaceuticals Inc.** Augustine M *US BANCORP PIPER JAFFRAY INC* 2001 September 27

428348 **Alexion Pharmaceuticals reports presentation of results from phase II rheumatoid arthritis trial at American College of Rheumatology meetings.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2001 November 14

434561 **Alexion completes phase I dermatomyositis pilot safety study.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2001 December 03

435653 **Complement (first of two parts).** Walport MJ *NEW ENGL J MED* 2001 **344** 14 1058-1066

435654 **Complement (second of two parts).** Walport MJ *N ENGL J MED* 2001 **344** 15 1140-1144

435656 **Controlling the complement system in inflammation.** Kirschfink M *IMMUNOPHARMACOLOGY* 1997 **38** 1-2 51-62

435694 **Complement activation and inhibition in experimental models of arthritis.** Linton SM, Morgan BP *MOL IMMUNOL* 1999 **36** 13-14 905-914

435718 **Complement and innate immunity.** Song WC, Sarma MR, Lambris JD *IMMUNOPHARMACOLOGY* 2000 **48** 1-2 17-25

435722 **Complement inhibitors: a resurgent concept in anti-inflammatory therapeutics.** Sahu A, Lambris JD *IMMUNOPHARMACOLOGY* 2000 **48** 1-2 133-148

435723 **C1 inhibitor in anti-inflammatory therapy: from animal experiment to clinical application.** Kirschfink M, Nurnberger W *MOL IMMUNOL* 1999 **36** 4-5 225-232

435868 **Alexion Pharmaceuticals Inc.** *HAMBRECHT & QUIST* 2002 January 7-10 18

435963 **Alexion completes enrollment in first of two acute myocardial infarction phase II trials with pexelizumab.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2002 January 15

437814 **Alexion starts phase IIb trial of SG1.1 in rheumatoid arthritis patients.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2002 January 29

438165 **Anti-inflammatory therapeutics (part II), London, UK.** Plasse TF *IDDB MEETING REPORT* 2002 January 14-15

440583 **Alexion completes enrollment in phase II membranous nephritis clinical trial with SG1.1.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2002 February 20

443717 **Alexion issued key C5 complement inhibitor patent for inflammatory diseases.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2002 March 18

445228 **The complement system.** Mayer MM *SCI AM* 1973 **229** 5 54-66

445230 **Arthritis critally dependent on innate immune system players.** Ji H, Ohmura K, Mahmood U, Lee DM, Hofhuis FM, Boackle SA, Takahashi K, Holers VM, Walport M, Gerard C, Ezekowitz A *et al IMMUNITY* 2002 **16** 2 157-168

445242 **The immunopathogenic role of complement in human muscle disease.** Mendell JR, Sahenk Z, Kissel JT *CURR OPIN NEUROL* 1996 **9** 3 226-234

445249 **Complement deposition in renal allografts with early malfunction.** Eggertsen G, Nyberg G, Nilsson B, Nilsson U, Svalander CT *APMIS* 2001 **109** 12 825-834

445251 **Complement plays an important role in gastric mucosal damage induced by ischemia-reperfusion in rats.** Jun T, Ikai M, Cerisna Y, Kunikawa T, Sano K, Yokoyama Y, Okada N, Itoh M *LIFE SCI* 2001 **70** 1 109-117

445254 **The role of the complement cascade in ischemia/reperfusion injury: implications for neuroprotection.** D'Ambrosio AL, Pinsky DJ, Connolly ES *MOL MED* 2001 **7** 6 367-382

445256 **The membrane attack complex of complement causes severe demyelination associated with acute axonal injury.** Mead RJ, Singhrao SK, Neal JW, Lassmann H, Morgan BP *J IMMUNOL* 2002 **1** 168 458-466

445272 **Activation of complement in psoriasis.** Rosenberg EW, Noah PW, Skinner RB *CLIN EXP DERMATOL* 1999 **24** 4 235

445278 **Complement in Alzheimer's disease: opportunities for modulating protective and pathogenic events.** Tenner AJ *NEUROBIOL AGING* 2001 **22** 5 849-861

445288 **Complement facilitates early prion pathogenesis.** Klein MA, Kaeser PS, Schwarz P, Weyd H, Xenarios I, Zinkernagel RM, Carroll MC, Verbeek JS, Botto M, Walport MJ, Molina H *et al NAT MED* 2001 **7** 4 488-492

445289 **Complement and its implications in cardiac ischemia/reperfusion: strategies to inhibit complement.** Mamsnjon T, Richard V, Fontaine M *FUNDAM CLIN PHARMACOL* 2001 **15** 5 295-306

445311 **Inhibition of complement, neutrophil, and platelet activation by an anti-factor D monoclonal antibody in simulated cardiopulmonary bypass circuits.** Fung M, Loubser PG, Undar A, Mueller M, Sun C, Sun WN, Vaughn WK, Fraser CD *J THORAC CARDIOVASC SURG* 2001 **112** 1 113-122

445313 **Inhibition of complement C5 reduces local and remote organ injury after intestinal ischemia/reperfusion in the rat.** Wada K, Montalto MC, Stahl GL *GASTROENTEROLOGY* 2001 **120** 126-133

445317 **A novel role of complement: mice deficient in the fifth component of complement (C5) exhibit impaired liver regeneration.** Mastellos D, Papadimitriou JC, Franchini S, Tsonis PA, Lambris JD *J IMMUNOL* 2002 **168** 4 2479-2486

445321 **A role for complement C5 in organism containment and granulomatous response during murine tuberculosis.** Actor JK, Brelji E, Wetsel RA, Hoffmann H, Hunter RL, Jagannath C *SCAND J IMMUNOL* 2001 **53** 5 464-474

445327 **Contribution of anaphylatoxin C5a to late airway responses after repeated exposure of antigen to allergic rats.** Abe M, Shibata K, Akatsu H, Shimizu N, Katsunagi T, Okada H *J IMMUNOL* 2001 **167** 4651-4660

445336 **C5a and C5a(desArg) enhance the susceptibility of monocyte-derived macrophages to HIV infection.** Kacani L, Banki Z, Zwirner J, Schennach H, Bajtay Z, Erdei A, Stoiber H, Dierich MP *J IMMUNOL* 2001 **166** 5 3410-3415

445340 **Activated human T lymphocytes express a functional C3a receptor.** Werfel T, Kirchhoff K, Wittmann M, Segerman G, Kapp A, Heidenreich F, Gotze O, Zwirner J *J IMMUNOL* 2000 **165** 11 6599-6605

445352 **Expression of the complement anaphylatoxin C3a and C5a receptors on bronchial epithelial and smooth muscle cells in models of sepsis and asthma.** Drouin SM, Kildsgaard J, Haviland J, Zabner J, Jia HP, McCray PB, Tack BF, Wetsel RA *J IMMUNOL* 2001 **166** 3 2025-2032

445354 **C3A binds to the seven transmembrane anaphylatoxin receptor expressed by epithelial cells and triggers the production of IL-8.** Mamsnjon T, Gasque P, Ischenko A, Fontaine M *FEBS LETT* 2001 **487** 3 339-346

445382 **Product pipeline.** Alexion Pharmaceuticals Inc *COMPANY WORLD WIDE WEB SITE* 2002 April 03 *http://www.alexionpharm.com/products/*

445384 **The membrane attack complex of complement causes severe demyelination associated with acute axonal injury.** Mead RJ, Singhrao SK, Neal JW, Lassmann NH, Morgan BP *J IMMUNOL* 2002 **168** 1 458-466

445786 **Complement.** Ruddy S *KELLEY'S TEXTBOOK RHEUMATOL (EDS RUDDY S HARRIS ED SLEDGE CB)* 2000 **6th Ed** 186-192

446377 **Alexion completes enrollment in second of two acute myocardial infarction phase II trials with pexelizumab.** Alexion Pharmaceuticals Inc *PRESS RELEASE* 2002 April 08

446660 **Alexion Pharmaceuticals Inc: the leader in complement inhibition.** Augustine ME *US BANCORP PIPER JAFFRAY INC* 2002 March

© 2006 International Society of Nephrology

New approaches to the treatment of glomerular diseases

GB Appel[1], M Waldman[1] and J Radhakrishnan[1]

[1]*Division of Nephrology, Department of Medicine, Columbia University College of Physicians and Surgeons, New York, New York, USA*

The treatment of primary glomerular diseases has previously focused on relatively toxic immunosuppressive regimens. We are now seeing a paradigm shift from these toxic 'nonspecific' therapies to selective immunomodulating and immunosuppressive regimens. In this article, the experience with these newer agents including mycophenolate, rituximab, sirolimus, calcineurin inhibitors, and eculizumab are described.

Kidney International (2006) **70,** S45–S50. doi:10.1038/sj.ki.5001977

KEYWORDS: glomerular disease; mycophenolate; rituximab; sirolimus; calcineurin inhibitors; eculizumab

Correspondence: *GB Appel, Division of Nephrology, Department of Medicine, Columbia University College of Physicians and Surgeons, New York, New York, USA. E-mail: gba2@columbia.edu*

In recent years, the therapy of glomerular diseases has dramatically changed. Certain 'nonspecific' interventions appear to be beneficial in almost all patients with renal diseases. These include optimizing blood pressure control to a level of no greater than 130/80 mm Hg and use of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers, either alone, or in combination. Recent data also suggests that HMG-CoA reductase inhibitors may decrease proteinuria and slow progression of renal disease. Although the data for dietary therapy remains more controversial, many clinicians utilize moderate protein restriction, low cholesterol/saturated fat, and low salt diets in their glomerular disease patients.

Despite the significant benefits of these non-immune modulating therapies, we are still in need of better immune-modulating drugs to treat glomerular diseases. In part, this is to control refractory disease, and in part to prevent relapses and flares. Patients who experience complete (or even partial) sustained remissions of proteinuria have less glomerular sclerosis, interstitial fibrosis, and slower progression to renal failure. Finally, there is a need to design effective immuno-suppressive strategies while avoiding the toxicities of conventional global immunosuppression.

The use of newer immune-modulating agents in a variety of glomerular disease remains to be defined. This is true despite some well-designed controlled randomized trials. In this manuscript, we review some of the recent studies examining the use of newer immunosuppressants in common glomerular diseases, including lupus nephritis, immunoglobulin A (IgA) nephropathy, focal glomerulo-sclerosis, and membranous nephropathy. We emphasize the value and shortcomings of these studies and describe new on-going trials.

LUPUS NEPHRITIS

In lupus nephritis (LN), advances in immunosuppressive treatment have occurred in recent years due to results from large, multicenter randomized controlled trials (RCT). Until recently, the standard treatment of severe LN consisted of 6 monthly pulses of IV cyclophosphamide (CYC) (0.5–1 g/m^2) followed by follow-up CYC pulses every 3 months based upon trials from the National Institutes of Health.[1–4] Some clinicians gave concomitant IV methylprednisolone based on data suggesting improved long-term renal survival without

Samsung Bioepis Exhibit No. 1002
Page 1244

additional toxicity. Despite considerable efficacy, this regimen was associated with short- and long-term adverse effects, and some patients failed to achieve remission or relapsed after treatment. Thus, major efforts have been taken to develop alternate immunosuppressive regimens that are equally effective but less toxic. Strategies include minimizing use of CYC with lower dosages, use of sequential therapies with different immunosuppressive agents and avoidance of CYC entirely with alternate agents. Although no specific regimen has become the new standard of care for LN, recent studies lend strong support to the use of alternate treatment regimens for both induction and maintenance regiments for proliferative LN.

The Euro Lupus Trial by Houssiau *et al.*[5] compared low-dose to conventional high-dose IV CYC as induction therapy for severe LN. Ninety patients, of whom 85% were white with class IV LN, were randomized to either standard doses CYC (6 monthly IV pulses followed by two quarterly pulses) or low-dose CYC (fixed pulses of 500 mg biweekly $\times$ 6) followed by maintenance with azathioprine (AZA). There was no significant difference in renal remission with high-dose CYC (54%) and low-dose CYC (71%) and renal flares occurred in 29 and 27%, respectively. There was a trend towards more infections with high-dose CYC. This trial suggests that a shorter course of lower dose CYC for induction followed by AZA may be an equally effective and less-toxic regimen. However, it is unclear whether these results are applicable to all high-risk patients as the study included few black patients, a population known to be a greater risk of progressive disease.[6,7]

Several major controlled trials have demonstrated the role of mycophenolate mofetil (MMF) as a promising therapeutic alternative for treatment of severe LN. A trial by Chan *et al.*[8] randomized 42 Chinese patients with DPLN to either MMF (induction with 2 g/day $\times$ 6 months, followed by maintenance with 1 g/day for 6 months) or 6 months induction with oral CYC (2.5 mg/kg/day) followed by maintenance with AZA for 6 months. At the end of 12 months, MMF and CYC were found to have equivalent efficacy with no differences in complete and partial remissions or relapses. Extended observation over 5 years with addition of 22 patients confirmed the long-term benefits of MMF with similar rate of relapse and relapse-free survival in both groups.[9] Adverse events including leukopenia, amenorrhea, and infections were more frequent in the CYC group and two deaths occurred in this group. This suggests a role for MMF in induction therapy for many Class IV lupus patients. Similar results have been confirmed by another trial of 46 Chinese patients treated with either MMF or pulse IV CYC for 6 months, which showed greater reductions in proteinuria, hematuria, anti-DNA antibody titers, and greater improvement on repeat renal biopsy in patients treated with MMF.[10]

A recently published multicenter RCT by Ginzler *et al.*[11] evaluated the efficacy of MMF in 140 patients with proliferative LN. In this study, 56% of the patients were African Americans with active urinary sediment, significant proteinuria, and active lupus serologies, thus representing a high-risk population. Patients were randomized to induction therapy with either standard 6 monthly pulses of IV CYC or MMF (target dose 3 g/day) in conjunction with a fixed tapering course of corticosteroids. The study allowed cross-over at 3 months for treatment failures. At six months, there were more complete remissions and complete plus partial remissions in the MMF arm. Side effect profile was also better in the MMF group with fewer severe infections. At median follow up of 3 years, there was no difference between treatment arms in number of patients with renal failure, end-stage renal disease or mortality, although all tended to be lower with MMF. A major limitation of the study is that it lacks long-term follow up. Furthermore, it is unclear if patients with more severe LN with crescents and necrosis will have a similar favorable response to MMF. Nevertheless, this study adds significantly to our experience with MMF in high risk, non-white patients. A large multicenter international randomized trial is now underway to further evaluate and compare induction therapy with either MMF or IV CYC (Aspreva Lupus Management Study) (abstract; Appel G *et al.* *J Am Soc Nephrol* 2005; **16**: 528A)

MMF was evaluated as maintenance therapy for proliferative LN in a study which randomized 59 patients to receive either MMF, AZA, or quarterly IV CYC after induction with standard monthly IV CYC pulses.[12] Fewer patients treated with AZA and MMF reached the primary end points of death and chronic renal failure (CRF) compared to the CYC group. Relapse-free survival was also higher with MMF and AZA. There was increased mortality in the IV CYC group. Hospitalizations, amenorrhea, infections, and gastrointestinal side effects were significantly lower in the MMF and AZA groups. A RCT (MAINTAIN Nephritis Trial) conducted by the European Working Party on Systemic Lupus Erythematosus is currently underway to compare the efficacy of MMF and AZA as maintenance treatment for proliferative LN following induction therapy with a short course of IV CYC.

Rituximab, a chimeric monoclonal antibody directed against the pan B-cell marker CD20 has been used to treat LN in numerous case reports and small open label uncontrolled trials. Rituximab was effective in inducing remission in some patients with severe LN, including some who were refractory to conventional immunosuppression.[13–15] In light of these promising results, the role of rituximab for LN is currently being investigated in a multicenter prospective placebo-controlled trial (LUNAR by Genetech) in which both arms of the study will receive MMF induction, but only one arm with receive concomitant rituximab.

Another novel targeted approach to the treatment of systemic lupus erythematosus involves blocking the interaction between T and B cells. The interaction of B7 molecules on B cells and CD28 on T cells provides an important co-stimulatory signal for T-cell activation and production of antibodies by B cells. Cytotoxic T lymphocyte antigen 4, a molecule structurally similar to CD28 on T cells has an

Samsung Bioepis Exhibit No. 1002
Page 1245

inhibitory effect on T-cell activation by preventing B7/CD28 interaction. Cytotoxic T lymphocyte antigen 4-Ig, a fusion molecule that combines cytotoxic T lymphocyte antigen 4 and an immunoglobulin chain, was developed to take advantage of this regulatory potential. In murine models of lupus, cytotoxic T lymphocyte antigen 4-Ig has been shown to reduce autoantibody production, attenuate glomerulonephritis and prolong survival. Although this approach has not yet been applied in human LN, clinical trials using these monoclonal antibodies are being formulated. Earlier clinical trials using other monoclonal antibodies (IDEC-131, BG9588) targeting another important receptor ligand pair, CD40/CD40L, have proven unsuccessful, in part due to lack of efficacy and in part due to thrombotic complications.[16–18]

IgA NEPHROPATHY

There are only a few well-designed randomized controlled trials evaluating different therapies in IgA nephropathy (IgAN). The evidence for some 'nonspecific therapies' such as optimizing blood pressure control, blockade of the rennin–angiotensin–aldosterone system seems clear. There is even controlled data supporting the use of combined blockade of the rennin–angiotensin system using ACE inhibitors and AII receptor antagonists concomitantly. The benefit of the remainder of therapies including fish oils, tonsillectomy, and immunosuppression for IgAN remains controversial.

A number of studies have evaluated the role of fish oil in IgAN, but have led to conflicting results. A RCT by Pettersson *et al.* in Sweden found that fish oils provided no renoprotective effects and actually led to a greater decline in GFR than placebo (corn oil).[19] In contrast, the RCT of 106 IgAN patients from the Mayo Clinic clearly showed a benefit of fish oils.[20] Fewer patients treated with fish oil compared to placebo had a 50% increase in plasma creatinine (6 vs 33%, respectively). The fish oil arm also showed a slower decline in GFR, less need for dialysis and transplantation, and fewer deaths.[21] However, a more recent RCT by Hogg *et al.* showed no benefit following 2 years of treatment with fish oil compared to corticosteroids or placebo (abstract; Hogg R *et al.*, *J Am Soc Nephrol* 2003; **14**: 751A). At present, given the contradictory results, it is reasonable to allow patients decide for themselves whether the side effects and cost of fish oil therapy outweigh potentially unproven renal or cardiovascular benefits.

The role of immunosuppressive therapy in IgAN remains unclear. A number of studies have examined the role of corticosteroids in patients with IgAN and led to mixed results. In one controlled trial from Japan, Katafuchi *et al.*[22] randomized 90 IgAN patients with baseline serum creatinine (Scr) <1.5 mg/dl to receive oral prednisone (induction with 20 mg, gradually tapered to 5 mg/day over 2 years) or placebo. Blood pressure control was tight, although rennin–angiotensin–aldosterone system blockade was not used. Proteinuria declined more in the steroid-treated group, but renal survival was identical (85%) at follow up (65 ± 25 months) in both groups. In contrast, in a large well-designed RCT from Italy by Pozzi *et al.*[23] 86 IgAN patients (baseline Scr <1.5 mg/dl and 1–3.5 g/day proteinuria) were randomized to placebo or corticosteroids (pulse methylprednisolone at the beginning of each month followed by alternate day corticosteroids for 6 months). After 10 years of follow up, renal survival was better in the steroid group compared to placebo (97 vs 53%, respectively).[24] The steroid regimen led to few side effects. It is unknown whether replacing the corticosteroid pulses with high dose oral corticosteroids would have similar efficacy. It is also unknown whether tighter blood pressure control and universal use of rennin–angiotensin–aldosterone system blockade in the subjects would have minimized any of the observed benefits of steroids. Nevertheless, this study suggests a role for a 6-month trial of corticosteroids in IgAN patients with proteinuria of >1 g/day, yet preserved renal function. A recent meta-analysis also supports the use of corticosteroids in reducing proteinuria and preventing progression to end-stage renal disease, although this study was not controlled for blood pressure or use of angiotensin-converting enzyme inhibitors.

The role of CYC was studied by Ballardie and Roberts.[25] in 38 patients at high risk of progression (i.e., abnormal serum creatinine, rising by at least 15% in the year before entry and end-stage renal disease predicted within 5 years). Patients were randomized to either placebo or oral CYC (1.5 mg/kg/day for 3 months) plus steroids followed by AZA (1.5 mg/kg/day) for 2 or more years. The 5-year renal survival in the treated group was 72 vs 6% in the control group. This study may be faulted for suboptimal blood pressure control, insufficient use of rennin–angiotensin–aldosterone system blockade, small number of patients, and unusually poor survival rate of the placebo group. Clearly, more supportive evidence is required before this treatment can be widely accepted but it may be reasonable to use this therapy in a subset of patients with significant proteinuria and deteriorating renal function. Other investigators have suggested a clear beneficial role of CYC in IgAN patients with true crescentic glomerulonephritis when compared to the course of historical controls.

The role of MMF for IgAN has been evaluated in four major trials with conflicting results. Chen X *et al.*[26] and Chen Z *et al.*[27] compared MMF vs prednisone in 62 Chinese patients with IgAN (mean urine protein >2 g/day) and showed a benefit with respect to proteinuria in the MMF treatment arm. Serum creatinine was higher in the prednisone group at 72 weeks. Tang *et al.*[28] also showed a greater reduction in proteinuria in patients treated with MMF compared to placebo, but there was no difference in serum creatinine. In contrast, Maes *et al.*[29] randomized 34 IgAN patients (mean serum creatinine 1.4 mg/dl, urine protein excretion 1.6 g/day) to either MMF (2 g daily) or placebo and found no significant differences in creatinine or proteinuria at 3 years follow-up. Similarly, we were unable to demonstrate benefit of MMF in IgAN patients with moderately

Samsung Bioepis Exhibit No. 1002
Page 1246

advanced disease in our single center-blinded RCT of MMF vs placebo.[30] Patients had a mean SCr of 2.4 mg/dl and mean urine protein excretion of 2.7 g/day and thus were at high risk of progressive renal disease. All patients had optimal BP control with angiotensin-converting enzyme inhibitors. This trial was prematurely terminated after enrolling only 40 patients due to the high rate of progression in both arms of the study. Thus, despite demonstrated benefit of MMF in other glomerular diseases, at present, there is insufficient evidence to support the use of MMF for the treatment of IgAN.

FOCAL SEGMENTAL GLOMERULOSCLEROSIS

Focal segmental glomerulosclerosis (FSGS) is the most common cause of the idiopathic nephrotic syndrome (NS) in adult African-Americans. It is also the most common primary glomerular disease leading to end-stage renal disease in the United States. Although there have been few controlled, randomized trials of immunosuppressive regimens in treating adults with FSGS, both a prolonged course of corticosteroids and cyclosporine (CSA) are considered effective in inducing remissions of the NS and in preventing progressive renal failure. Cyclosporine has been shown to be more effective than placebo in an RCT in adult FSGS in which all patients were steroid resistant and 40% had failed cytotoxic therapy.[31] In the CSA arm, 70% of patients achieved a complete or partial remission of the NS vs only 4% of the placebo group. In addition, at 4 years follow up, fewer patients treated with CSA had a 50% decrease in GFR compared to placebo (25 vs 52%, respectively). Thus, CSA has proven to be an effective treatment for adults with FSGS. There is far less data on MMF in the treatment of FSGS.

One study by Choi *et al.*[32] examined the use of MMF in 18 FSGS patients (including 12 with renal insufficiency and nine with the NS), who were either steroid resistant or steroid/cyclosporine dependent. Following treatment with MMF and for many, concurrent steroids, the urine protein to creatinine ratio decreased to 48%, two patients achieved complete remission and six had partial remission of the NS. A second study examined the course of 18 steroid-resistant FSGS patients after MMF therapy.[33] All patients had the NS (mean proteinuria 9.1 g/day) and 75% of patients had also failed therapy with a cytotoxic agent and/or a calcineurin inhibitor previously. After 6 months of MMF, although there were no complete remissions, six had partial remissions, two had a 50% reduction in proteinuria, and two had a decrease in proteinuria below the nephrotic range. There were no changes in serum creatinine over the study period, and there was no significant toxicity from MMF. Currently there is a multicenter, RCT sponsored by the National Institutes of Health, comparing MMF to CSA in patients aged 3–40 years with steroid-resistant FSGS.

Sirolimus is widely used in renal transplantation. It was hoped that it would provide the efficacy of calcineurin inhibitors without significant nephrotoxicity. Data on its use in FSGS are conflicting. Two small uncontrolled, non-randomized trials suggest episodes of acute renal failure with this agent in FSGS patients (abstract: Cho M *et al.*, *J Am Soc Nephol* 2005; **16**: 775A). A third trial of 21 patients found major reductions in proteinuria and correction of sieving coefficient defects in a group of FSGS patients treated with sirolimus. (Tumlin *et al.* CJASN 2006) At present, with concerns of proteinuria in transplant patients treated with this drug, more data will be needed before it can be recommended for new trials treating FSGS in native kidneys.

MEMBRANOUS NEPHROPATHY

Membranous nephropathy (MN) remains the most common pattern of idiopathic NS in Caucasian adults.[34] It appears that only certain patients will benefit from immunosuppressive treatment, whereas others will do well over the long term with only 'nonspecific therapy'.[35] In general, patients with heavy persistent nephrotic range proteinuria, older female and male subjects, and those with renal dysfunction appear to benefit from the treatment.[36–39] A host of therapies have been recommended, but only a few have been investigated in RCTs. Cytotoxic agents such as chlorambucil and cyclosphosphamide have been shown to be effective in reducing proteinuria in patients with MN (Ponticelli C, Zucchelli P, Passerini P *et al.* A 10-year follow-up of a randomized study with methylprednisolone and chlorambucil in membranous nephropathy. *Kidney Int* 1995; **48**: 1600–1604; Ponticelli C, Altieri P, Scolari F *et al.* A randomized study comparing methylprednisolone plus chlorambucil versus methylprednisolone plus cyclophosphamide in idiopathic membranous nephropathy. *J Am Soc Nephrol* 1998; **9**: 444–450; du Buf-Vereijken PW, Branten AJ, Wetallzelthis JF: Membranous Nephropathy Study Group. Cytotoxic therapy for membranous nephropathy and renal insufficiency: improved renal survival but high relapse rate. *Nephrol Dial Transplant* 2004; **19**: 1142–1148).

Cyclosporine has been shown to induce more complete and partial remissions of the NS in a randomized trial in which 51 patients were randomized to either CSA plus low-dose steroids vs steroids alone.[40] At the end of 6 months of treatment, 21 of 28 vs 5 of 23 placebo-treated patients were in partial or complete remission. The remission rate remained significant in favor of CSA at longer follow up, although few patients from either group progressed to renal failure.

MMF has been used in a series of uncontrolled trials in high-risk nephrotic patients with MN.[32,41] In 17 MN, patients treated with MMF, Choi *et al.*[32] reported a decrease in median urine protein/creatinine ratio from 7.3 (0.1, 18.5) to 1.5 ($<$0.1, 14.3) ($P = 0.001$), without a significant change in serum creatinine. In a study by Miller *et al.*,[41] 16 MN patients who had failed therapy with other immunosuppressive agents were treated for a mean duration of 8 months with MMF. Six patients had a $\geqslant$50% reduction in proteinuria.

Eculizumab is a humanized monoclonal antibody that prevents the activation of complement component C5. In a randomized, placebo-controlled trial in 200 patients with

Samsung Bioepis Exhibit No. 1002
Page 1247

MN, eculizumab although well-tolerated, failed to show any significant reduction of proteinuria. There were concerns that the dosing schedules were inadequate as inhibition of complement was not uniformly demonstrated. (Appel G, Nachman P, Hogan S *et al.* Eculizumab (C5 complement inhibitor) in the treatment of idiopathic membranous nephropathy [Abstract]. *J Am Soc Nephrol* 2002; **13**: 668A–1857).

Rituximab was recently shown to reduce proteinuria in small uncontrolled trials of patients with MN.[42,43] In eight nephrotic patients with MN, Ruggenenti *et al.* studied the effects of treatment with Rituximab. CD20 cells remained depleted for up to 12 months. Five of the eight patients had a slow but progressive decline in proteinuria to subnephrotic levels over the first 6 months, and the three other patients had a smaller decline in proteinuria. This effect on proteinuria remained for up to 1 year after treatment. Adverse effects were rare. In a later analysis by the same group, rituximab was found to be effective only in those patients without interstitial fibrosis and/or significant renal dysfunction at the time of biopsy (abstract: Ruggenenti P *et al.*, *J Am Soc Nephrol* 2003; **14**: 528A).

It is clear that there is a need for newer, effective yet safe, immunosuppressive agents to treat a variety of glomerular diseases. Some new therapies have been adopted from use in transplantation, whereas others are derived from experiences in the oncology or rheumatologic arenas. Fortunately, many are being studied in a randomized, controlled fashion. These trials should lead to more specifically targeted treatment strategies for patients with glomerular diseases in the near future.

ACKNOWLEDGMENTS

This study was supported by The Glomerular Center at Columbia University.

REFERENCES

1. Austin III HA, Klippel JH, Balow JE *et al.* Therapy of lupus nephritis. Controlled trial of prednisone and cytotoxic drugs. *N Engl J Med* 1986; **314**: 614–619.
2. Boumpas DT, Austin III HA, Vaughn EM *et al.* Controlled trial of pulse methylprednisolone versus two regimens of pulse cyclophosphamide in severe lupus nephritis. *Lancet* 1992; **340**: 741–745.
3. Gourley MF, Austin III HA, Scott D *et al.* Methylprednisolone and cyclophosphamide, alone or in combination, in patients with lupus nephritis. A randomized, controlled trial. *Ann Intern Med* 1996; **125**: 549–557.
4. Illei GG, Austin HA, Crane M *et al.* Combination therapy with pulse cyclophosphamide plus pulse methylprednisolone improves long-term renal outcome without adding toxicity in patients with lupus nephritis. *Ann Intern Med* 2001; **135**: 248–257.
5. Houssiau FA, Vasconcelos C, D'Cruz D *et al.* Immunosuppressive therapy in lupus nephritis: the Euro-Lupus Nephritis Trial, a randomized trial of low-dose versus high-dose intravenous cyclophosphamide. *Arthritis Rheum* 2002; **46**: 2121–2131.
6. Austin III HA, Boumpas DT, Vaughan EM *et al.* High-risk features of lupus nephritis: importance of race and clinical and histological factors in 166 patients. *Nephrol Dial Transplant* 1995; **10**: 1620–1628.
7. Barr RG, Seliger S, Appel GB *et al.* Prognosis in proliferative lupus nephritis: the role of socio-economic status and race/ethnicity. *Nephrol Dial Transplant* 2003; **18**: 2039–2046.
8. Chan TM, Li FK, Tang CS *et al.* Efficacy of mycophenolate mofetil in patients with diffuse proliferative lupus nephritis. Hong Kong-Guangzhou Nephrology Study Group. *N Engl J Med* 2000; **343**: 1156–1162.
9. Chan TM, Tse KC, Tang CS *et al.* Long-term study of mycophenolate mofetil as continuous induction and maintenance treatment for diffuse proliferative lupus nephritis. *J Am Soc Nephrol* 2005; **16**: 1076–1084.
10. Hu W, Liu Z, Chen H *et al.* Mycophenolate mofetil vs cyclophosphamide therapy for patients with diffuse proliferative lupus nephritis. *Chin Med J (Engl)* 2002; **115**: 705–709.
11. Ginzler EM, Dooley MA, Aranow C *et al.* Mycophenolate mofetil or intravenous cyclophosphamide for lupus nephritis. *N Engl J Med* 2005; **353**: 2219–2228.
12. Contreras G, Pardo V, Leclercq B *et al.* Sequential therapies for proliferative lupus nephritis. *N Engl J Med* 2004; **350**: 971–980.
13. Leandro MJ, Cambridge G, Edwards JC *et al.* B-cell depletion in the treatment of patients with systemic lupus erythematosus: a longitudinal analysis of 24 patients. *Rheumatology (Oxford)* 2005; **44**: 1542–1545.
14. Looney RJ, Anolik JH, Campbell D *et al.* B cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalation trial of rituximab. *Arthritis Rheum* 2004; **50**: 2580–2589.
15. Sfikakis PP, Boletis JN, Lionaki S *et al.* Remission of proliferative lupus nephritis following B cell depletion therapy is preceded by down-regulation of the T cell costimulatory molecule CD40 ligand: an open-label trial. *Arthritis Rheum* 2005; **52**: 501–513.
16. Boumpas DT, Furie R, Manzi S *et al.* A short course of BG9588 (anti-CD40 ligand antibody) improves serologic activity and decreases hematuria in patients with proliferative lupus glomerulonephritis. *Arthritis Rheum* 2003; **48**: 719–727.
17. Kalunian KC, Davis Jr JC, Merrill JT *et al.* Treatment of systemic lupus erythematosus by inhibition of T cell costimulation with anti-CD154: a randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* 2002; **46**: 3251–3258.
18. Davis Jr JC, Totoritis MC, Rosenberg J *et al.* Phase I clinical trial of a monoclonal antibody against CD40-ligand (IDEC-131) in patients with systemic lupus erythematosus. *J Rheumatol* 2001; **28**: 95–101.
19. Pettersson EE, Rekola S, Berglund L *et al.* Treatment of IgA nephropathy with omega-3-polyunsaturated fatty acids: a prospective, double-blind, randomized study. *Clin Nephrol* 1994; **41**: 183–190.
20. Donadio Jr JV, Bergstralh EJ, Offord KP *et al.* A controlled trial of fish oil in IgA nephropathy. Mayo Nephrology Collaborative Group. *N Engl J Med* 1994; **331**: 1194–1199.
21. Donadio Jr JV, Grande JP, Bergstralh EJ *et al.* The long-term outcome of patients with IgA nephropathy treated with fish oil in a controlled trial. Mayo Nephrology Collaborative Group. *J Am Soc Nephrol* 1999; **10**: 1772–1777.
22. Katafuchi R, Ikeda K, Mizumasa T *et al.* Controlled, prospective trial of steroid treatment in IgA nephropathy: a limitation of low-dose prednisolone therapy. *Am J Kidney Dis* 2003; **41**: 972–983.
23. Pozzi C, Bolasco PG, Fogazzi GB *et al.* Corticosteroids in IgA nephropathy: a randomised controlled trial. *Lancet* 1999; **353**: 883–887.
24. Pozzi C, Andrulli S, Del Vecchio L *et al.* Corticosteroid effectiveness in IgA nephropathy: long-term results of a randomized, controlled trial. *J Am Soc Nephrol* 2004; **15**: 157–163.
25. Ballardie FW, Roberts IS. Controlled prospective trial of prednisolone and cytotoxics in progressive IgA nephropathy. *J Am Soc Nephrol* 2002; **13**: 142–148.
26. Chen X, Chen P, Cai G *et al.* A randomized control trial of mycophenolate mofeil treatment in severe IgA nephropathy. *Zhonghua Yi Xue Za Zhi* 2002; **82**: 796–801.
27. Chen X, Wu J, Zhang Y *et al.* 72 weeks follow up study of the effects of mycophenolate mofetil on IgA nephropathy (abstract). *J Am Soc Nephrol* 2001; **12**: 66A.
28. Tang S, Leung JC, Chan LY *et al.* Mycophenolate mofetil alleviates persistent proteinuria in IgA nephropathy. *Kidney Int* 2005; **68**: 802–812.
29. Maes BD, Oyen R, Claes K *et al.* Mycophenolate mofetil in IgA nephropathy: results of a 3-year prospective placebo-controlled randomized study. *Kidney Int* 2004; **65**: 1842–1849.
30. Frisch G, Lin J, Rosenstock J *et al.* Mycophenolate mofetil (MMF) vs placebo in patients with moderately advanced IgA nephropathy: a double-blind randomized controlled trial. *Nephrol Dial Transplant* 2005; **20**: 2139–2145.
31. Cattran DC, Appel GB, Hebert LA *et al.* A randomized trial of cyclosporine in patients with steroid-resistant focal segmental glomerulosclerosis. North America Nephrotic Syndrome Study Group. *Kidney Int* 1999; **56**: 2220–2226.

Samsung Bioepis Exhibit No. 1002
Page 1248

32. Choi MJ, Eustace JA, Gimenez LF *et al*. Mycophenolate mofetil treatment for primary glomerular diseases. *Kidney Int* 2002; **61**: 1098–1114.
33. Cattran DC, Wang MM, Appel G *et al*. Mycophenolate mofetil in the treatment of focal segmental glomerulosclerosis. *Clin Nephrol* 2004; **62**: 405–411.
34. Haas M, Meehan SM, Karrison TG *et al*. Changing etiologies of unexplained adult nephrotic syndrome: a comparison of renal biopsy findings from 1976–1979 and 1995–1997. *Am J Kidney Dis* 1997; **30**: 621–631.
35. Schieppati A, Mosconi L, Perna A *et al*. Prognosis of untreated patients with idiopathic membranous nephropathy. *N Engl J Med* 1993; **329**: 85–89.
36. Reichert LJ, Koene RA, Wetzels JF. Prognostic factors in idiopathic membranous nephropathy. *Am J Kidney Dis* 1998; **31**: 1–11.
37. Wu Q, Jinde K, Nishina M *et al*. Analysis of prognostic predictors in idiopathic membranous nephropathy. *Am J Kidney Dis* 2001; **37**: 380–387.
38. Pei Y, Cattran D, Greenwood C. Predicting chronic renal insufficiency in idiopathic membranous glomerulonephritis. *Kidney Int* 1992; **42**: 960–966.
39. Cattran DC, Pei Y, Greenwood CM *et al*. Validation of a predictive model of idiopathic membranous nephropathy: its clinical and research implications. *Kidney Int* 1997; **51**: 901–907.
40. Cattran DC, Appel GB, Hebert LA *et al*. Cyclosporine in patients with steroid-resistant membranous nephropathy: a randomized trial. *Kidney Int* 2001; **59**: 1484–1490.
41. Miller G, Zimmerman III R, Radhakrishnan J *et al*. Use of mycophenolate mofetil in resistant membranous nephropathy. *Am J Kidney Dis* 2000; **36**: 250–256.
42. Remuzzi G, Chiurchiu C, Abbate M *et al*. Rituximab for idiopathic membranous nephropathy. *Lancet* 2002; **360**: 923–924.
43. Ruggenenti P, Chiurchiu C, Brusegan V *et al*. Rituximab in idiopathic membranous nephropathy: a one-year prospective study. *J Am Soc Nephrol* 2003; **14**: 1851–1857.

Samsung Bioepis Exhibit No. 1002
Page 1249

ClinicalTrials.gov

A service of the U.S. National Institutes of Health

Try our beta test site

69 studies found for: eculizumab

Modify this search | How to Use Search Results

List | By Topic | On Map | Search Details

Show Display Options Download Subscribe to RSS

Only show open studies

Rank	Status	Study
41	Terminated	Safety & Efficacy of Eculizumab to Prevent AMR in Living Donor Kidney Transplant Recipients Requiring Desensitization **Condition:** Antibody Mediated Rejection **Intervention:** Drug: Eculizumab **Study Start:** September 2011 **First Received:** July 19, 2011
42	Withdrawn	Targeting Complement Activation in Antineutrophil Cytoplasmic Autoantibodies (ANCA)-Vasculitis - Eculizumab **Condition:** Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis **Interventions:** Drug: Standard of care treatment; Drug: eculizumab **Study Start:** May 2011 **First Received:** December 13, 2010
43	Completed	Safety and Efficacy Study of Eculizumab in Shiga-Toxin Producing Escherichia Coli Hemolytic-Uremic Syndrome (STEC-HUS) **Condition:** Shiga-like Toxin-producing Escherichia Coli **Intervention:** Drug: Eculizumab (Soliris®) **Study Start:** July 2011 **First Received:** August 3, 2011
44	Completed	Extension Study of Eculizumab in Patients With Transfusion Dependent Paroxysmal Nocturnal Hemoglobinuria (PNH) **Condition:** Paroxysmal Hemoglobinuria, Nocturnal **Intervention:** Drug: eculizumab **Study Start:** May 2005 **First Received:** July 20, 2005
45	Recruiting	Complement Inhibition Using Eculizumab to Overcome Platelet Transfusion Refractoriness in Patients With Severe Thrombocytopenia **Conditions:** Thrombocytopenia; Alloimmune Platelet Refractoriness **Intervention:** Drug: Eculizumab **Study Start:** November 4, 2014 **First Received:** November 21, 2014
46	Recruiting	Eculizumab in Shiga-toxin Related Hemolytic and Uremic Syndrome Pediatric Patients - ECULISHU **Condition:** Hemolytic Uremic Syndrome of Childhood **Interventions:** Drug: Eculizumab; Drug: Placebo **Study Start:** June 2015 **First Received:** July 28, 2014
47	Completed	Eculizumab to Treat Paroxysmal Nocturnal Hemoglobinuria

Condition: Hemoglobinuria, Paroxysmal
Intervention: Drug: Eculizumab
Study Start: December 2004
First Received: December 3, 2004

---

48 Completed — Eculizumab to Treat Paroxysmal Nocturnal Hemoglobinuria
Condition: Paroxysmal Hemoglobinuria, Nocturnal
Intervention: Drug: Eculizumab
Study Start: August 9, 2005
First Received: August 11, 2005

---

49 Completed — Study Using Eculizumab in Transfusion Dependent Paroxysmal Nocturnal Hemoglobinuria (PNH) Patients
Condition: Hemoglobinuria, Paroxysmal
Intervention: Drug: eculizumab
Study Start: October 2004
First Received: July 18, 2005

---

50 Completed — Study of Safety in Hemolytic Paroxysmal Nocturnal Hemoglobinuria (PNH) Patients Treated With Eculizumab
Condition: Hemoglobinuria, Paroxysmal
Intervention: Drug: eculizumab
Study Start: December 2004
First Received: July 19, 2005

---

51 Completed — Eculizumab in Treating Patients With Paroxysmal Nocturnal Hemoglobinuria
Condition: Leukemia
Intervention: Biological: eculizumab
Study Start: November 2004
First Received: June 2, 2005

---

52 Completed — Safety and Efficacy Study of Eculizumab in Patients With Mild Allergic Asthma
Condition: Allergic Asthma
Intervention: Drug: eculizumab
Study Start: September 2007
First Received: June 12, 2007

---

53 Completed — A Retrospective, Observational, Non-interventional Trial to Assess Eculizumab Treatment Effect in Patients With Atypical Hemolytic Uremic Syndrome (aHUS)
Condition: Atypical Hemolytic Uremic Syndrome (aHUS)
Intervention:
Study Start: August 2009
First Received: November 13, 2012

---

54 Unknown † — The Safety and Efficacy of Eculizumab in Japanese Patients With Atypical Hemolytic Uremic Syndrome (aHUS)
Condition: Atypical Hemolytic Uremic Syndrome (aHUS)
Intervention: Drug: Eculizumab
Study Start: May 2012
First Received: December 4, 2012

---

55 Completed — Safety and Effectiveness of h5G1.1-mAb for Dermatomyositis
Condition: Dermatomyositis
Intervention: Drug: h5G1.1-mAb
Study Start: April 2000
First Received: April 25, 2000

---

56 Completed — To Characterize the Safety and Tolerability of Eculizumab in Two Japanese aHUS Patients
Condition: ATYPICAL HEMOLYTIC UREMIC SYNDROME (AHUS)
Intervention:
Study Start: May 2012
First Received: December 4, 2012

Samsung Bioepis Exhibit No. 1002
Page 1251

57	Not yet recruiting	Phase 2 Safety and Efficacy Study of RA101495 to Treat PNH Patients Who Have an Inadequate Response to Eculizumab **Condition:** Paroxysmal Nocturnal Hemoglobinuria (PNH) **Intervention:** Drug: RA101495 **Study Start:** February 2017 **First Received:** January 20, 2017
58	Recruiting	Identification and Treatment of Thrombotic Microangiopathies in Allogeneic Stem Cell Transplants **Conditions:** Thrombotic Microangiopathy; Disorder Related to Bone Marrow Transplantation **Intervention:** Drug: eculizumab **Study Start:** September 2014 **First Received:** October 15, 2015
59	Active, not recruiting	Paroxysmal Nocturnal Hemoglobinuria (PNH) Registry **Condition:** Paroxysmal Nocturnal Hemoglobinuria **Intervention:** **Study Start:** January 2007 **First Received:** April 15, 2011
60	Terminated	aHUS Observational Long Term Follow-Up **Condition:** Atypical Hemolytic Uremic Syndrome **Intervention:** **Study Start:** March 2012 **First Received:** January 18, 2012

TO TOP

⏮ ◀ Previous Page (21-40) Studies Shown (41-60) Next Page (61-69) ▶ ⏭

† Study has passed its completion date and status has not been verified in more than two years.

Samsung Bioepis Exhibit No. 1002
Page 1252

ORIGINAL ARTICLE

Effect of Eculizumab on Hemolysis and Transfusion Requirements in Patients with Paroxysmal Nocturnal Hemoglobinuria

Peter Hillmen, M.B., Ph.D., Claire Hall, M.B., Ch.B., Judith C.W. Marsh, M.B., M.D., Modupe Elebute, M.B., M.D., Michael P. Bombara, B.S., Beth E. Petro, B.S., Matthew J. Cullen, B.Sc., Stephen J. Richards, Ph.D., Scott A. Rollins, Ph.D., Christopher F. Mojcik, M.D., Ph.D., and Russell P. Rother, Ph.D.

ABSTRACT

BACKGROUND

Paroxysmal nocturnal hemoglobinuria (PNH) arises from a somatic mutation of the *PIG-A* gene in a hematopoietic stem cell and the subsequent production of blood cells with a deficiency of surface proteins that protect the cells against attack by the complement system. We tested the clinical efficacy of eculizumab, a humanized antibody that inhibits the activation of terminal complement components, in patients with PNH.

METHODS

Eleven transfusion-dependent patients with PNH received infusions of eculizumab (600 mg) every week for four weeks, followed one week later by a 900-mg dose and then by 900 mg every other week through week 12. Clinical and biochemical indicators of hemolysis were measured throughout the trial.

RESULTS

Mean lactate dehydrogenase levels decreased from 3111 IU per liter before treatment to 594 IU per liter during treatment (P=0.002). The mean percentage of PNH type III erythrocytes increased from 36.7 percent of the total erythrocyte population to 59.2 percent (P=0.005). The mean and median transfusion rates decreased from 2.1 and 1.8 units per patient per month to 0.6 and 0.0 units per patient per month, respectively (P=0.003 for the comparison of the median rates). Episodes of hemoglobinuria were reduced by 96 percent (P<0.001), and measurements of the quality of life improved significantly.

CONCLUSIONS

Eculizumab is safe and well tolerated in patients with PNH. This antibody against terminal complement protein C5 reduces intravascular hemolysis, hemoglobinuria, and the need for transfusion, with an associated improvement in the quality of life in patients with PNH.

From the Department of Haematology, Leeds Teaching Hospitals National Health Science Trust, Leeds, United Kingdom (P.H., C.H., M.J.C., S.J.R.); the Department of Haematology, St. George's Hospital Medical School, London (J.C.W.M., M.E.); and Alexion Pharmaceuticals, Cheshire, Conn. (M.P.B., B.E.P., S.A.R., C.F.M., R.P.R.). Address reprint requests to Dr. Hillmen at the Department of Haematology, Leeds General Infirmary, Great George St., Leeds LS1 3EX, United Kingdom, or at peter.hillmen@panp-tr.northy.nhs.uk.

N Engl J Med 2004;350:552-9.

Copyright © 2004 Massachusetts Medical Society.

The New England Journal of Medicine
Downloaded from nejm.org at US PATENT & TRADEMARK OFFICE on March 28, 2017. For personal use only. No other uses without permission.
Copyright © 2004 Massachusetts Medical Society. All rights reserved.

Samsung Bioepis Exhibit No. 1002
Page 1253

THE MAJOR CLINICAL SIGNS OF PAROXysmal nocturnal hemoglobinuria (PNH) are intravascular hemolysis, venous thrombosis, and hemoglobinuria.[1] The disease arises from a somatic mutation of the *PIG-A* gene in a pluripotent hematopoietic stem cell. *PIG-A* encodes a protein that is essential for the synthesis of glycosylphosphatidylinositol (GPI), a lipid moiety that is embedded in the plasma membrane, where it serves to anchor a wide variety of proteins to the cell surface. The mutant stem cell subsequently expands to form a hematopoietic clone with a deficiency in proteins that are normally attached to the cell surface by the GPI anchor.[2,3] The mature blood cells derived from the hematopoietic clone can have a complete deficiency (type III) or a partial deficiency (type II) of GPI-linked proteins and almost always coexist with residual cells with a normal expression of these proteins (previously identified as type I).

The clinical features of PNH result from the lack of one or more GPI-linked proteins that protect cells from complement-mediated attack. Two such proteins — CD55 and CD59 — are absent from PNH type III erythrocytes, platelets, and other blood cells.[4-6] CD55 regulates early complement activation by inhibiting C3 convertases,[7] whereas CD59 inhibits the assembly of the membrane-attack complex C5b–C9 by interacting with C8 and C9.[4,5] The lack of CD59 is probably responsible for the increased sensitivity of PNH erythrocytes and platelets to complement.[4,8-13]

Eculizumab is a recombinant humanized monoclonal antibody that was designed to block the activation of terminal complement components.[14,15] It binds specifically to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b, thereby preventing the release of the inflammatory mediator C5a and the formation of the cytolytic-pore C5b–C9. Blockade of the complement cascade at C5 preserves the early components of complement that are essential for the opsonization of microorganisms and clearance of immune complexes.[16] In this trial, we investigated whether eculizumab could reduce the incidence of intravascular hemolysis, hemoglobinuria, and transfusion requirements in patients with PNH.

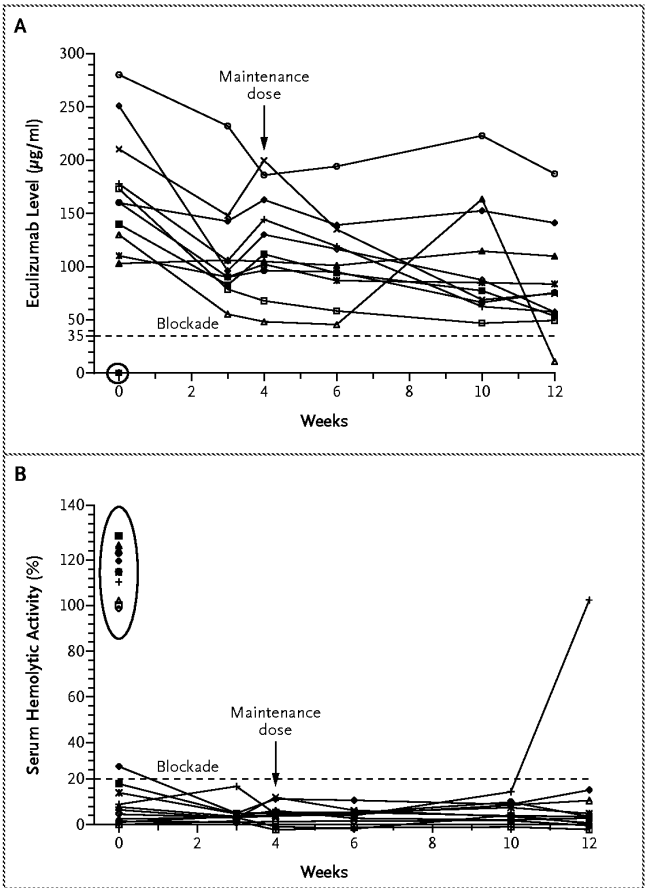


Figure 1. Pharmacokinetic and Pharmacodynamic Analysis of Eculizumab in 11 Patients with Paroxysmal Nocturnal Hemoglobinuria (PNH).

Panel A depicts serum levels of eculizumab during the 12-week treatment period. Eculizumab levels just before a patient's first dose of eculizumab are circled on the x axis. Also shown at time zero are levels of eculizumab one hour after the initial dose. Other data points indicate trough levels of eculizumab for each patient at weeks 3, 4, 6, 10, and 12. The beginning of the 900-mg maintenance dose is indicated by an arrow. The level of eculizumab required to block complement activity (≥35 μg per milliliter) is indicated by a dashed line. Panel B shows hemolytic activity in serum during the 12-week treatment period, as assessed in a presensitized chicken-erythrocyte hemolytic assay. Pretreatment serum hemolytic activity values are circled at time zero. Also shown at time zero are values for hemolytic activity one hour after the initial dose. Other data points indicate trough hemolytic activity at weeks 3, 4, 6, 10, and 12. The dashed line indicates the level of hemolytic activity that is considered to represent complete complement blockade (≤20 percent).

METHODS

PATIENTS

The study was conducted from May through December 2002. Men and women (18 years of age and older) who had received a diagnosis of PNH at least six months earlier, had a detectable GPI-deficient hematopoietic clone, and had received at least four red-cell transfusions in the preceding 12 months were

The New England Journal of Medicine
Downloaded from nejm.org at US PATENT & TRADEMARK OFFICE on March 28, 2017. For personal use only. No other uses without permission.
Copyright © 2004 Massachusetts Medical Society. All rights reserved.

Samsung Bioepis Exhibit No. 1002
Page 1254

eligible. Patients were required to have a negative throat culture for *Neisseria meningitidis* and *N. gonorrhoeae*. All patients were vaccinated against *N. meningitidis* (Mengivac (A+C), Aventis Pasteur) before treatment. One patient had a stroke after consent but never received eculizumab and was excluded. Patients who were taking stable doses of immunosuppressive drugs (e.g., cyclosporine), warfarin, and iron supplements were permitted to continue them.

The trial was approved by the local research ethics committee and was performed according to the International Conference on Harmonisation and Good Clinical Practice Standards. Eleven patients gave written informed consent and were treated with eculizumab.

TREATMENT SCHEDULE

Patients received infusions of 600 mg of eculizumab weekly for four weeks, followed one week later by a 900-mg dose and then by a dose of 900 mg every other week through week 12.

INVESTIGATIONS

In this open-label pilot study, we obtained data on the pharmacokinetics, pharmacodynamics, and immunogenicity of eculizumab and observed its clinical effects by measuring the following: lactate dehydrogenase, haptoglobin, bilirubin, and hemoglobin levels; reticulocyte counts; the proportion of GPI-deficient cells, as assessed by flow cytometry[17]; the rate of transfusion with packed red cells; the rate of occurrence of hemoglobinuria (assessed by daily comparison of the first morning urine sample with a standardized color chart before and during treatment); and the quality of life, as reflected by the scores on the European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30 instrument. The trigger for transfusion during the study period remained unchanged for each patient, as compared with their care before entry into the study: patients received blood transfusions when they had symptoms resulting from anemia.

Assessment of the safety of eculizumab included ascertainment of treatment-related adverse events, electrocardiography, and routine laboratory tests (e.g., serum chemical analyses and complete blood counts).

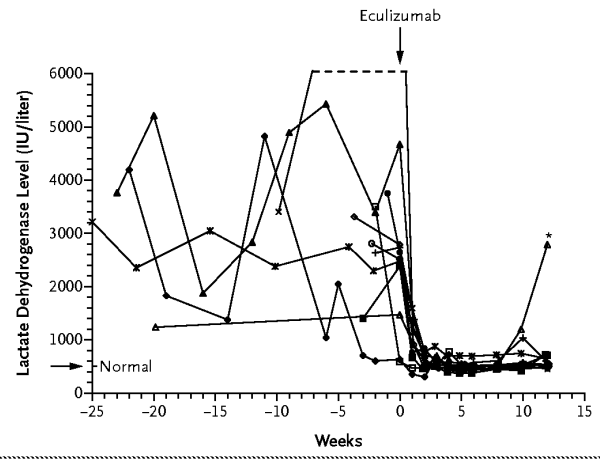


Figure 2. Analysis of Lactate Dehydrogenase Levels, a Biochemical Indicator of Hemolysis, in 11 Patients with Paroxysmal Nocturnal Hemoglobinuria up to 25 Weeks before and during 12 Weeks of Eculizumab Treatment.

The first dose of eculizumab is indicated by an arrow, as is the upper limit of the normal range of lactate dehydrogenase at the Leeds Teaching Hospital. The data point identified at week 12 by the asterisk represents a reading that was obtained from a duplicate serum sample since the original sample was lost. The dashed line represents off-scale points from one patient with a peak value of 12,100 IU per liter.

ASSAY METHODS

The pharmacokinetics of eculizumab were determined with an enzyme-linked immunosorbent assay that detects both free and C5-bound eculizumab.[15] The pharmacodynamics of eculizumab were determined by measuring the capacity of the patient's serum to lyse chicken erythrocytes in a standard total human serum-complement hemolytic assay.[18] The presence or absence of antibodies against eculizumab was assessed by an enzyme-linked immunosorbent assay.[19]

STATISTICAL ANALYSIS

Biochemical values were compared with the use of a paired Student's t-test, quality-of-life measurements with the use of a mixed-effect analysis of covariance, the median rate of transfusions with the use of a Wilcoxon signed-rank test, and the comparison of the number of days with paroxysms with the use of Fisher's exact test.

The corresponding author and the sponsor were jointly responsible for the design of this trial and the development of the protocol. Data were collected and analyzed by a clinical research organization, Kendle International, which maintained the trial data base and provided statistical support. The manuscript was prepared by the corresponding author, with substantial review and comments by the other authors and the sponsor. Final decisions on

The New England Journal of Medicine
Downloaded from nejm.org at US PATENT & TRADEMARK OFFICE on March 28, 2017. For personal use only. No other uses without permission.
Copyright © 2004 Massachusetts Medical Society. All rights reserved.

Samsung Bioepis Exhibit No. 1002
Page 1255

the content of the manuscript rested with the corresponding author in consultation with the other authors. All authors had access to the primary data.

RESULTS

DEMOGRAPHIC CHARACTERISTICS

Six men and five women (median age, 48 years; range, 21 to 67) with a median duration of PNH of 8.6 years (range, 1.7 to 37.4) participated in the trial. Five of the patients had platelet counts at base line of less than 150,000 per cubic millimeter. Eight patients had previously received a diagnosis of aplastic anemia, two were concomitantly receiving cyclosporine for aplastic anemia, and six were receiving warfarin.

SAFETY

All patients completed the 12-week study. There were no deaths or thrombotic events, and all patients subsequently entered a 12-month extension study. Each patient reported one or more adverse events during the trial. Events reported by three patients included headache and upper respiratory tract infection. Events reported by two patients included influenza-like symptoms, rigors, dizziness, nausea, nasal congestion, and joint aches. None of these events were attributed to the study medication. Serious adverse events occurred in two patients. The first was hospitalized with a viral chest infection. The second reported nausea, vomiting, and headache after the first infusion, with dizziness and shivering the following day. The patient was hospitalized overnight, and subsequent infusions were well tolerated.

PHARMACOKINETICS, PHARMACODYNAMICS, AND IMMUNOGENICITY OF ECULIZUMAB

Peak and trough levels of eculizumab were well above 35 μg per milliliter from one hour after the first dose through the completion of the 600-mg weekly dose period (Fig. 1A). In 10 patients, serum trough levels of eculizumab remained above 35 μg per milliliter for the entire study.

The hemolytic activity of serum from these 10 patients was completely blocked (less than 20 percent in the chicken–red-cell assay) for essentially the entire treatment period (Fig. 1B). In 1 of the 11 patients, the trough level of eculizumab fell below 35 μg per milliliter at week 12 and serum hemolytic activity returned. In no case were antibodies against eculizumab detected.

BIOCHEMICAL INDICATORS OF HEMOLYSIS

Levels of lactate dehydrogenase in serum were markedly elevated in all patients before eculizumab treatment. Mean (±SD) lactate dehydrogenase levels fell from 3111±598 IU per liter during the 12 months before enrollment to 594±32 IU per liter (normal range, 150 to 480) during treatment ($P=0.002$) (Fig. 2).

The decrease in lactate dehydrogenase began after a single dose of eculizumab in all patients. Lactate dehydrogenase levels remained within or just above the normal range for the duration of the study (Fig. 2). In the one patient in whom eculizumab levels fell below 35 μg per milliliter at week 12 (Fig. 1A), hemolytic activity returned (Fig. 1B) and lactate dehydrogenase levels increased transiently (Fig. 2).

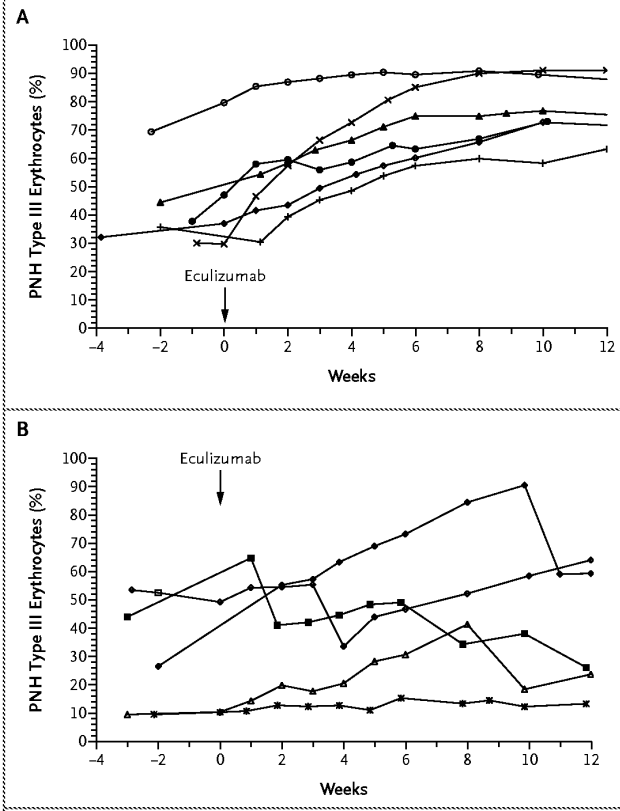


Figure 3. Changes in the Percentage of Paroxysmal Nocturnal Hemoglobinuria (PNH) Type III Erythrocytes during Eculizumab Treatment in Six Patients Who Received No Transfusions during Treatment (Panel A) and Five Patients Who Received at Least One Transfusion after Starting Treatment (Panel B).
The initial dose of eculizumab is indicated by the arrow in each panel.

The New England Journal of Medicine
Downloaded from nejm.org at US PATENT & TRADEMARK OFFICE on March 28, 2017. For personal use only. No other uses without permission.
Copyright © 2004 Massachusetts Medical Society. All rights reserved.

Samsung Bioepis Exhibit No. 1002
Page 1256

The dosing frequency was increased from 900 mg every 14 days to 900 mg every 12 days, reestablishing complete complement blockade during the ongoing 12-month extension study (data not shown).

Haptoglobin became detectable in the serum of 5 of the 11 patients after two weeks of eculizumab treatment but returned to undetectable levels soon thereafter (data not shown). Bilirubin levels were also elevated in most patients at base line and did not change significantly during treatment (data not shown).

EFFECT ON PNH CLONES

Type III erythrocytes are highly sensitive to lysis by complement and as a result have a short life span. In our study, the percentage of type III erythrocytes increased significantly from a mean of 36.7±5.9 percent before treatment to 59.2±8.0 percent at the end of 12 weeks of treatment (P=0.005) (Fig. 3). The increase was particularly consistent in six patients who remained transfusion-independent during the study (Fig. 3A). In patients who received transfusions during the study, sudden drops in the proportion of type III cells were seen as the transfused red cells diluted the type III population (Fig. 3B). There were no significant changes in the percentages of PNH type III neutrophils, monocytes, or platelets during treatment with eculizumab; in most cases, these percentages were 90 to 100 percent before the study (data not shown).

TRANSFUSION REQUIREMENTS, HEMOGLOBIN LEVELS, AND RETICULOCYTE COUNTS

During the year preceding enrollment, the range of red-cell transfusions received by the 11 patients was 12 to 55 units, whereas during the three months of the study, the range was 0 to 8 units. Before eculizumab treatment, the mean and median transfusion rates were 2.1 and 1.8 units per patient per month, respectively (Table 1). These transfusion rates decreased to 0.6 and 0.0 unit per patient per month, respectively, during the three months of treatment with eculizumab (P=0.003). Hemoglobin levels did not increase significantly during the treatment period, although hemoglobin values in six patients stabilized without transfusions (Table 1). Similarly, the numbers of reticulocytes remained relatively constant during eculizumab treatment.

HEMOGLOBINURIA

Our patients recorded the color of their urine each morning using a color chart designed to assess the

Table 1. **Transfusion Rates, Hemoglobin Levels, and Reticulocyte Counts 12 Months before and after 3 Months of Eculizumab Treatment.**

Patient No.	12 Mo before Eculizumab Treatment				After 3 Mo of Eculizumab Treatment			
	Transfusions		Hemoglobin	Reticulocytes	Transfusions		Hemoglobin	Reticulocytes
	no. of units	*rate**	*g/dl*	*×10⁻³/mm³*	*no. of units*	*rate†*	*g/dl*	*×10⁻³/mm³*
1	22	1.8	10.3	77.5	2	0.7	10.0	100.7
2	23	1.9	8.3	200.0	8	2.9	8.8	182.6
3	20	1.6	10.1	169.5	0	0.0	10.7	175.9
4	28	2.3	9.3	282.0	0	0.0	9.4	333.3
5	12	1.0	11.9	96.3	2	0.7	10.6	121.8
6	14	1.2	9.8	346.8	0	0.0	10.6	259.0
7	34	2.8	12.8	100.6	0	0.0	13.5	166.8
8	21	1.7	9.5	164.5	0	0.0	9.8	239.6
9	55	4.5	10.7	138.0	3	1.1	11.4	285.8
10	41	3.4	8.5	108.7	5	1.8	8.8	140.1
11	14	1.2	8.5	91.4	0	0.0	10.0	97.4
Median‡		1.8				0.0		
Mean		2.1	10.0	161.4		0.6	10.3	191.2

* The rate (in units per month) was calculated as (number of units÷365 days)×30.
† The rate (in units per month) was calculated as (number of units÷84 days)×30.
‡ P=0.003 for the change in the median rate of transfusion by the Wilcoxon signed-rank test.

The New England Journal of Medicine
Downloaded from nejm.org at US PATENT & TRADEMARK OFFICE on March 28, 2017. For personal use only. No other uses without permission.
Copyright © 2004 Massachusetts Medical Society. All rights reserved.

Samsung Bioepis Exhibit No. 1002
Page 1257

degree of hemoglobinuria (Fig. 4A) during both the 2-to-4-week screening period and the 12-week treatment period. Paroxysms of hemoglobinuria were prospectively defined as dark-colored urine with a colorimetric level of 6 or more. In nine patients for whom urine scores were assessed, the mean incidence of paroxysms was reduced from 2.9 days to 0.12 day per patient per month ($P<0.001$) (Fig. 4B).

QUALITY OF LIFE

The quality of life was assessed with the use of the EORTC QLQ-C30 instrument. When responses at base line were compared with responses during 12 weeks of eculizumab treatment, there were significant improvements in the domains of global health status ($P=0.02$), physical functioning ($P<0.001$), emotional functioning ($P<0.001$), cognitive functioning ($P=0.002$), fatigue ($P<0.001$), dyspnea ($P=0.002$), and insomnia ($P=0.049$) (Table 2).

DISCUSSION

Patients with PNH have chronic, often disabling symptoms of fatigue and intermittent episodes of dysphagia, abdominal pain, and hemoglobinuria. These symptoms are thought to be related to the intravascular destruction of PNH type III erythrocytes, which are deficient in complement inhibitors, by autologous complement. The hemolytic anemia frequently renders the patients transfusion-dependent. In addition, patients have an extremely high risk of potentially life-threatening thrombosis, particularly thrombosis of the hepatic and cerebral veins. Approximately 50 percent of patients with PNH die of the disease; the median duration of survival after diagnosis is 10 years.[1]

We found that the defect in the membrane-bound inhibitor of terminal complement components in PNH was ameliorated by the administration of eculizumab. This antibody specifically prevents cleavage of C5, which is necessary for assembly of the membrane-attack complex. Blockade of terminal complement components presumably prolongs the survival of type III erythrocytes (since there was no simultaneous increase in reticulocytes), which are highly sensitive to lysis by complement, thereby increasing the proportion of these cells in the blood and reducing signs of hemolysis in most patients. In some patients, the percentage of type III erythrocytes increased to more than 80 percent of the total erythrocyte population. This interpretation of the mechanism of action of eculizumab is consistent with the report of an asymptomatic patient with PNH who had more than 80 percent type III erythrocytes and a concomitant deficiency in the terminal complement protein C9.[20] Thus, inhibition of the assembly of C5b–C9 by an antibody or by a congenital deficiency of a terminal component of the complement system can protect type III erythrocytes from complement-mediated lysis.

The long-term effects of protecting PNH type III erythrocytes from complement are not known. For example, will removing the negative pressure

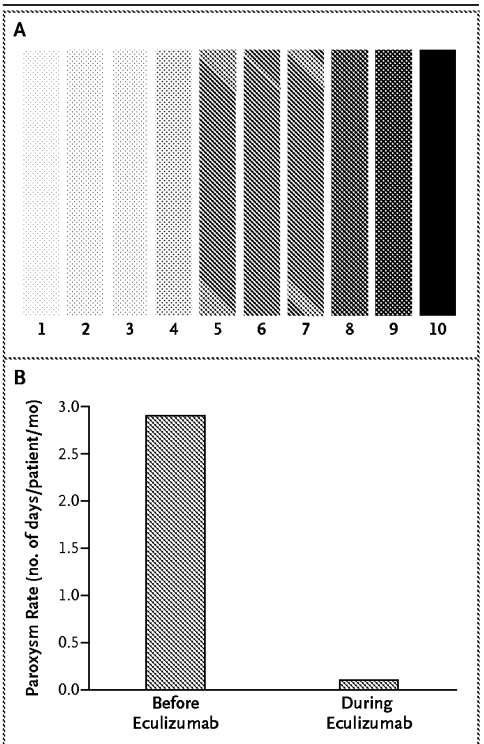


Figure 4. **Incidence of Paroxysms during Eculizumab Treatment.**

Panel A shows a urine color scale devised to monitor the incidence of paroxysms of hemoglobinuria in patients with paroxysmal nocturnal hemoglobinuria before and during treatment. A paroxysm was prospectively defined as a urine score of 6 or greater in this study. Panel B depicts the change in the paroxysm rate (defined as the mean number of days in paroxysm per patient per month) in nine patients 1 month before and during 12 weeks of eculizumab therapy. Pretreatment data on paroxysms were inadvertently not collected for two patients, and these two were therefore excluded from the analysis.

The New England Journal of Medicine
Downloaded from nejm.org at US PATENT & TRADEMARK OFFICE on March 28, 2017. For personal use only. No other uses without permission.
Copyright © 2004 Massachusetts Medical Society. All rights reserved.

Samsung Bioepis Exhibit No. 1002
Page 1258

Table 2. **Change in the Quality of Life during Eculizumab Treatment.***

Domain	Mean Base-Line Score†	Change from Base Line‡§	P Value§
Global health status	56.1	13.7	0.02
Physical functioning	70.9	13.0	<0.001
Emotional functioning	70.5	12.7	<0.001
Cognitive functioning	77.3	11.8	0.002
Fatigue	47.5	−15.3	<0.001
Dyspnea	39.4	−12.4	0.002
Insomnia	30.3	−10.8	0.049

* The quality of life was assessed with the European Organization for Research and Treatment of Cancer QLQ-C30 instrument.
† Numbers represent mean values of linearly transformed scores.
‡ Values for change from base line represent least-square means. A positive value indicates an improvement in the score for global health status, physical functioning, emotional functioning, and cognitive functioning, whereas a negative value indicates an improvement in the score for fatigue, dyspnea, and insomnia.
§ Values are from a mixed analysis-of-covariance model with visit as a fixed effect, patient as a random effect, and base line as a covariate.

on type III hematopoietic cells alter the rate of expansion of the PNH clone? What might occur in a patient with an increased population of PNH type III erythrocytes if treatment with eculizumab is stopped? Two of the patients who entered the eculizumab extension study had transient breakthroughs (lasting two to three days) in complement blockade until the dosing interval was adjusted. Both patients had hemoglobinuria with mild symptoms, but the episodes were not life threatening and were easily managed (data not shown). Definitive answers to these questions will require further study.

In this trial, lactate dehydrogenase levels declined rapidly and remained reduced as long as the serum level of eculizumab exceeded 35 μg per milliliter. The importance of maintaining this level of antibody was demonstrated in a single patient, in whom the eculizumab level transiently dropped below 35 μg per milliliter at week 12, resulting in a return of serum complement activity and an increase in lactate dehydrogenase levels. Subsequent administration of eculizumab reestablished complement blockade and rapidly reduced lactate dehydrogenase levels. Interestingly, lactate dehydrogenase levels were reduced in most patients to just above the upper limit of normal. The slightly elevated levels of this enzyme during treatment with eculizumab could reflect persistent, low-level C3b-mediated extravascular hemolysis or, possibly, undefined mechanisms of hemolysis that are unrelated to complement.

We also found that eculizumab treatment significantly reduced transfusion requirements, even though the levels of hemoglobin did not change significantly. However, the hemoglobin level in an individual patient before study entry was artificially maintained as a result of the transfusion of normal red cells. The transfused red cells survive far longer than PNH cells. Therefore, the stabilization of hemoglobin levels with a reduced need or no need for transfusion is a result of the protection of PNH red cells from complement-mediated lysis by eculizumab.

The decrease in transfusion requirements was most apparent in the six patients without a clinically significant degree of bone marrow failure (as defined by a normal platelet count). All but one of these patients were transfusion-independent during the study and remained so during an extension study. The remaining patient received a single 3-unit transfusion during the study, as compared with the receipt of 55 units in the 12 months preceding enrollment.

There was a rapid improvement in the quality of life during eculizumab therapy, as measured by the EORTC QLQ-C30. These clinical observations support the hypothesis that many of the important coexisting clinical conditions in patients with PNH are directly related to chronic and acute episodes of hemolysis, possibly through the scavenging of nitric oxide by plasma free hemoglobin.[21-25]

Eculizumab was safe and well tolerated during this open-label pilot study. The adverse events reported by patients were similar in type and frequency to those reported with either eculizumab or placebo in other controlled trials. All patients are currently participating in a one-year extension study in which the drug continues to be well tolerated. Furthermore, the rates of intravascular hemolysis, as measured by lactate dehydrogenase levels and hemoglobinuria, remain reduced in all patients, with 5 of 11 patients having been transfusion-independent for at least one year since starting eculizumab treatment.

The *PIG-A* mutation in patients with PNH causes deficiencies in the membrane-bound complement inhibitors CD55 and CD59, resulting in intravascular hemolysis.[2,3] However, patients who have genetic deficiencies in the surface expression of CD55 (Inab phenotype) with normal levels of CD59 have no clinical signs of hemolysis.[8,9] Conversely, a pa-

The New England Journal of Medicine
Downloaded from nejm.org at US PATENT & TRADEMARK OFFICE on March 28, 2017. For personal use only. No other uses without permission.
Copyright © 2004 Massachusetts Medical Society. All rights reserved.

Samsung Bioepis Exhibit No. 1002
Page 1259

tient with a genetic deficiency in the expression of CD59 but normal levels of CD55 had symptoms indistinguishable from those of PNH.[10,11] Therefore, the somatic mutation in the *PIG-A* gene that causes the deficiency of the membrane-bound terminal complement inhibitor CD59 is critical to the pathogenesis of PNH. We found that terminal complement inhibition with eculizumab ameliorated the untoward effects of this deficiency.

In summary, eculizumab appears to enhance the survival of type III PNH erythrocytes, improving the quality of life and reducing the extent of hemolysis, hemoglobinuria (the clinical hallmark of PNH), and the need for blood transfusions in patients with PNH. This study confirms that terminal complement activation is the key mediator of erythrocyte destruction in PNH.

Funded by Alexion Pharmaceuticals, Cheshire, Conn.

Dr. Hillmen reports serving as a consultant to Alexion Pharmaceuticals and receiving grant support from the company; Drs. Rollins, Mojcik, and Rother, Mr. Bombara, and Ms. Petro report having equity ownership in Alexion Pharmaceuticals; and Drs. Rollins and Rother have assigned to Alexion Pharmaceuticals their inventions made as employees of the company and have received no royalties from Alexion for these inventions. Dr. Rollins receives royalties for inventions he made before becoming an employee of Alexion.

We are indebted to Drs. Leonard Bell, Stephen Squinto, and Paul Finnegan for critical review of the manuscript; to Karen Hannon for excellent technical support; and to Jan Farish, David Buchanon, Pilar Hernandez-Campo, and the nurses of Healthcare-at-Home for their assistance in carrying out the study.

REFERENCES

1. Hillmen P, Lewis SM, Bessler M, Luzzatto L, Dacie JV. Natural history of paroxysmal nocturnal hemoglobinuria. N Engl J Med 1995;333:1253-8.
2. Takeda J, Miyata T, Kawagoe K, et al. Deficiency of the GPI anchor caused by a somatic mutation of the PIG-A gene in paroxysmal nocturnal hemoglobinuria. Cell 1993;73:703-11.
3. Bessler M, Mason PJ, Hillmen P, et al. Paroxysmal nocturnal hemoglobinuria (PNH) is caused by somatic mutations in the PIG-A gene. EMBO J 1994;13:110-7.
4. Holguin MH, Fredrick LR, Bernshaw NJ, Wilcox LA, Parker CJ. Isolation and characterization of a membrane protein from normal human erythrocytes that inhibits reactive lysis of the erythrocytes of paroxysmal nocturnal hemoglobinuria. J Clin Invest 1989;84:7-17.
5. Rollins SA, Sims PJ. The complement-inhibitory activity of CD59 resides in its capacity to block incorporation of C9 into membrane C5b-9. J Immunol 1990;144:3478-83.
6. Morgan BP. Isolation and characterization of the complement-inhibiting protein CD59 antigen from platelet membranes. Biochem J 1992;282:409-13.
7. Fujita T, Inoue T, Ogawa K, Iida K, Tamura N. The mechanism of action of decay-accelerating factor (DAF): DAF inhibits the assembly of C3 convertases by dissociating C2a and Bb. J Exp Med 1987;166:1221-8.
8. Merry AH, Rawlinson VI, Uchikawa M, Daha MR, Sim RB. Studies on the sensitivity to complement-mediated lysis of erythrocytes (Inab phenotype) with a deficiency of DAF (decay accelerating factor). Br J Haematol 1989;73:248-53.
9. Telen MJ, Green AM. The Inab phenotype: characterization of the membrane protein and complement regulatory defect. Blood 1989;74:437-41.
10. Yamashina M, Ueda E, Kinoshita T, et al. Inherited complete deficiency of 20-kilodalton homologous restriction factor (CD59) as a cause of paroxysmal nocturnal hemoglobinuria. N Engl J Med 1990;323:1184-9.
11. Motoyama N, Okada N, Yamashina M, Okada H. Paroxysmal nocturnal hemoglobinuria due to hereditary nucleotide deletion in the HRF20 (CD59) gene. Eur J Immunol 1992;22:2669-73.
12. Holguin MH, Martin CB, Bernshaw NJ, Parker CJ. Analysis of the effects of activation of the alternative pathway of complement on erythrocytes with an isolated deficiency of decay accelerating factor. J Immunol 1992;148:498-502.
13. Okada N, Harada R, Okada H. Erythrocytes of patients with paroxysmal nocturnal haemoglobinuria acquire resistance to complement attack by purified 20-kD homologous restriction factor. Clin Exp Immunol 1990;80:109-13.
14. Riechmann L, Clark M, Waldmann H, Winter G. Reshaping human antibodies for therapy. Nature 1988;332:323-7.
15. Thomas TC, Rollins SA, Rother RP, et al. Inhibition of complement activity by humanized anti-C5 antibody and single-chain Fv. Mol Immunol 1996;33:1389-401.
16. Matis LA, Rollins SA. Complement specific antibodies: designing novel anti-inflammatories. Nat Med 1995;1:839-42.
17. Richards SJ, Hillmen P. Advances in the laboratory diagnosis of paroxysmal nocturnal hemoglobinuria. Clin Appl Immunol Rev 2001;1:315-30.
18. Rinder CS, Rinder HM, Smith BR, et al. Blockade of C5a and C5b-9 generation inhibits leukocyte and platelet activation during extracorporeal circulation. J Clin Invest 1995;96:1564-72.
19. Colnot DR, Roos JC, de Bree R, et al. Safety, biodistribution, pharmacokinetics, and immunogenicity of 99mTc-labeled humanized monoclonal antibody BIWA 4 (bivatuzumab) in patients with squamous cell carcinoma of the head and neck. Cancer Immunol Immunother 2003;52:576-82.
20. Yonemura Y, Kawakita M, Koito A, et al. Paroxysmal nocturnal haemoglobinuria with coexisting deficiency of the ninth component of complement: lack of massive haemolytic attack. Br J Haematol 1990;74:108-13.
21. Rosse WF. Paroxysmal nocturnal hemoglobinuria. In: Hoffman R, Benz EJ Jr, Shattil SJ, et al., eds. Hematology: basic principles and practice. 3rd ed. New York: Churchill Livingstone, 2000:331-42.
22. Murray JA, Ledlow A, Launspach J, Evans D, Loveday M, Conklin JL. The effects of recombinant human hemoglobin on esophageal motor functions in humans. Gastroenterology 1995;109:1241-8.
23. Przybelski RJ, Daily EK, Kisicki JC, Mattia-Goldberg C, Bounds MJ, Colburn WA. Phase I study of the safety and pharmacologic effects of diaspirin cross-linked hemoglobin solution. Crit Care Med 1996;24:1993-2000.
24. Savitsky JP, Doczi J, Black J, Arnold JD. A clinical safety trial of stroma-free hemoglobin. Clin Pharmacol Ther 1978;23:73-80.
25. Corbin JD, Francis SH, Webb DJ. Phosphodiesterase type 5 as a pharmacologic target in erectile dysfunction. Urology 2002;60:Suppl 2:4-11.

Copyright © 2004 Massachusetts Medical Society.

The New England Journal of Medicine
Downloaded from nejm.org at US PATENT & TRADEMARK OFFICE on March 28, 2017. For personal use only. No other uses without permission.
Copyright © 2004 Massachusetts Medical Society. All rights reserved.

Samsung Bioepis Exhibit No. 1002
Page 1260

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15148839	BELL ET AL.
	Examiner	Art Unit
	JAMES ROGERS	1644

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
STN: DISSABS MEETINGS BIOSCI CAPLUS MEDICINE	8/8/2016	JR
EAST: PGPUB, USPAT, JPO, EPO, INVENTOR SEARCH	8/8/2016	JR
updated	3/2/2017	JR

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

	/James Rogers/ Examiner Art Unit 1644

ClinicalTrials.gov
A service of the U.S. National Institutes of Health
Try our beta test site

Trial record **1 of 1** for: nct00005571
Previous Study | Return to List | Next Study

Safety and Effectiveness of h5G1.1-mAb for Dermatomyositis

This study has been completed.

Sponsor:
National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)

Information provided by:
National Institutes of Health Clinical Center (CC)

ClinicalTrials.gov Identifier:
NCT00005571

First received: April 25, 2000
Last updated: March 3, 2008
Last verified: December 2001
History of Changes

Full Text View | Tabular View | No Study Results Posted | Disclaimer | How to Read a Study Record

Purpose

This study will evaluate the safety and effectiveness of the experimental drug h5G1.1-mAb in treating patients with dermatomyositis. This disease, which causes skin rash, muscle weakness, and sometimes various other symptoms, may be due to an immune system abnormality. Drugs currently used to treat dermatomyositis, such as prednisone and various anticancer drugs, often have serious side effects and may not work in all patients. h5G1.1-mAb is a genetically engineered antibody that blocks the activity of certain proteins involved in the immune reaction that produces inflammation.

Patients age 18 years and older who have had dermatomyositis for at least 6 months and who have not improved with prednisone or other therapies, or who cannot tolerate prednisone or other therapies, may be eligible for this 12-week study. Candidates will have a history and physical examination, including blood and urine tests, throat culture, and muscle strength testing. Participants will be randomly assigned to receive either h5G1.1-mAb or placebo (an inactive substance). The drug or placebo will be given intravenously (through a thin tube inserted into a vein) once a week for five doses and then every other week for two more doses.

Participants will undergo the following additional tests at various intervals during the study as follows:

1. Complete physical examination (visit 9)
2. Blood and urine tests (various intervals)
3. Muscle strength testing, assessment of ability to perform daily tasks, and completion of questionnaire regarding functional abilities (visits 2, 6 and 9)
4. Ultrasound imaging of muscle (during certain muscle exercises) (visits 2, 6 and 9)
5. Electrocardiogram (EKG) (visits 2 and 9)
6. Throat swab (culture) (visit 6)
7. Examination and photography of skin lesions (visits 2 and 9)
8. Skin biopsy - removal of small sample of skin tissue under local anesthetic (visits 2 and 9)
9. Magnetic resonance imaging (MRI) scan of muscles (visits 2 and 9)
10. Possible muscle biopsy - removal of small sample of muscle tissue under local anesthetic (visits 2 and 9).

Condition	Intervention	Phase
Dermatomyositis	Drug: h5G1.1-mAb	Phase 2

Study Type: Interventional
Study Design: Primary Purpose: Treatment

Official Title: A Randomized, Third-Party-Blind, Placebo-Controlled Pilot Study of the Effect of h5G1.1-mAb on Dermatomyositis Patients

Resource links provided by NLM:

Drug information available for: Eculizumab

Genetic and Rare Diseases Information Center resources: Idiopathic Inflammatory Myopathy Dermatomyositis Polymyositis

U.S. FDA Resources

Further study details as provided by National Institutes of Health Clinical Center (CC):

Estimated Enrollment:	17
Study Start Date:	April 2000
Estimated Study Completion Date:	December 2001

Detailed Description:

This is a randomized, third-party, placebo-controlled pilot study of h5G1.1-mAb, an antibody directed at the complement component C5, administered to patients with dermatomyositis with persistent disease. We are one of the four groups taking part in a multicenter trial under the sponsorship of Alexion Pharmaceuticals, Inc.

In dermatomytosis, humorally-mediated damage to muscle and skin microvasculature appears very important. The deposition of the membrane attack complex (C5-9) on the capillaries has been shown to precede the destruction of muscle fibers and to be a specific finding in the skin lesion. These observations, along with the recent discovery of the effectiveness of intravenous gammaglobulin (IVIG) in dermatomyositis, support the role of complement activation in the pathogenesis of dermatomyositis. Among the activated components of the complement system, the products that are generated after cleavage of C5, namely, C5a and C5b-9, are potent inflammatory mediators with pleiotropic activities. Inhibition of complement activation at C5 would prevent the formation of these pro-inflammatory molecules while allowing the generation of C3b, which is critical for opsonization and immune complex clearance. C5 inhibition therefore represents a potentially effective therapeutic modality for dermatomyositis.

Anti-C5 monoclonal antibodies are designed to prevent the cleavage of C5. A murine monoclonal antibody to human C5, m5G1.1-mAb, was humanized (h5G1.1-mAb) by grafting the antibody's antigen-binding CDR regions onto human antibody-derived framework and constant domains. This antibody is being made available by Alexion Pharmaceuticals, and will be used under their IND.

There will be 15 patients entered (with the goal of 12 evaluable patients) distributed among two treatment groups. Patients will be randomized at a 3 to 1 ratio between h5G1.1-mAb and placebo. Following a screening period of no less than 7 days, a 2 month double-blind, randomized treatment period will commence. Patients will receive an active (h5G1.1-mAb, 8mg/kg) bolus infusion every week X 5, then every 2 weeks X 2, or a placebo bolus infusion every week X 5, then every 2 weeks X 2. All patients will be receiving 7 infusions of h5G1.1-mAb or placebo.

The objective is to determine the safety and tolerability of h5G1.1-mAb and to evaluate the pharmacokinetics and pharmacodynamics of this antibody as well as its efficacy in the target population. Safety will be assessed via the collection of adverse events and the evaluation of pre- and post-treatment laboratory data. Efficacy will be assessed by the change in the manual muscle testing score relative to placebo at 2.25 months (one week following the end of the active treatment period) (primary efficacy value), and the change in the muscle enzyme level, MRI findings, skin examination findings and biopsy, and SF-36 (secondary efficacy values).

Eligibility

Ages Eligible for Study:	Child, Adult, Senior
Sexes Eligible for Study:	All
Accepts Healthy Volunteers:	No

Criteria

Patients age greater than or equal to 18 years.

Patients with a diagnosis of definite or probable dermatomyositis by criteria of Bohan et. al., with either active rash typical of dermatomyositis, history of rash typical of dermatomyositis, or Gottron's papules.

Patients with a manual muscle testing score less than or equal to 136/170.

Patients with a disease duration greater than or equal to 6 months.

Patients with persistent disease (defined as active rash plus CK greater than or equal to 2 times ULN), or rapidly progressive disease, or response to steroids with inability to taper dose, or unacceptable side effects of steroids.

Patients may be on stable (times 28 days prior to Visit 2) dose of MTX or AZA.

No other immunosuppressive agents times 84 days prior to first dose.

Patients on stable oral steroid use for 28 days prior to Visit 2.

Patients with adequate hematologic function, defined as hemoglobin greater than or equal to 8.5 g/dl, WBC greater than or equal to 3,000 mm(3), neutrophils greater than or equal to 1,200 mm(3), platelets greater than or equal to 100,000 mm(3).

Patients must be willing and able to give informed consent.

Women must be post-menopausal, surgically sterile or practicing a medically approved method of contraception.

Patients with liver disease will be excluded.

Patients with history of alcohol or drug abuse within two years of screening, or a history of positive Hepatitis B or Hepatitis C serology, unless vaccinated will be excluded.

Samsung Bioepis Exhibit No. 1002
Page 1263

Patients with renal insufficiency, defined as creatinine greater than or equal to 2.0 mg/dl will be excluded.

Patients with history of malignancy, except basal cell carcinoma and remote (greater than or equal to 5 years) malignancies in complete remission will be excluded.

Patients with history of poorly controlled diabetes will be excluded.

Patients with presence or suspicion of active infection, recent serious infection, or chronic/recurrent viral or bacterial infection will be excluded.

Patients with throat culture positive for pathogenic Neisseria species (meningitidis or gonorrhoeae) will be exluded. Subjects with a positive culture may be treated with appropriate antibiotic therapy and retested.

Patients with joint disease or replacement that would interfere with patient's ability to perform muscle testing will be excluded.

Patients with any clinically significant medical condition that is likely to interfere with the participation in the study or the evaluation of the study medication's safety profile will be excluded.

No patients with known or suspected hereditary complement deficiency will be excluded.

Patients with history of allergic reaction to murine proteins will be excluded.

Patients participating in any other investigational drug trial, or exposure to other investigational agent or device within thirty days prior to screening will be excluded.

Pregnant or breastfeeding patients will be excluded.

Women intending to conceive during the course of the study, including follow-up period will be excluded.

Patients with history of HIV, Lyme disease, or other environmentally induced myositis will be excluded.

Contacts and Locations

Choosing to participate in a study is an important personal decision. Talk with your doctor and family members or friends about deciding to join a study. To learn more about this study, you or your doctor may contact the study research staff using the Contacts provided below. For general information, see Learn About Clinical Studies.

Please refer to this study by its ClinicalTrials.gov identifier: NCT00005571

Locations

United States, Maryland

National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)
Bethesda, Maryland, United States, 20892

Sponsors and Collaborators

National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)

More Information

Publications:

Pearson CM, Bohan A. The spectrum of polymyositis and dermatomyositis. Med Clin North Am. 1977 Mar;61(2):439-57. Review.

Love LA, Leff RL, Fraser DD, Targoff IN, Dalakas M, Plotz PH, Miller FW. A new approach to the classification of idiopathic inflammatory myopathy: myositis-specific autoantibodies define useful homogeneous patient groups. Medicine (Baltimore). 1991 Nov;70(6):360-74.

Adams EM, Pucino F, Yarboro C, Hicks JE, Thornton B, McGarvey C, Sonies BC, Bartlett ML, Villalba ML, Fleisher T, Plotz PH. A pilot study: use of fludarabine for refractory dermatomyositis and polymyositis, and examination of endpoint measures. J Rheumatol. 1999 Feb;26(2):352-60.

ClinicalTrials.gov Identifier:	NCT00005571 History of Changes
Other Study ID Numbers:	000117 00-AR-0117
Study First Received:	April 25, 2000
Last Updated:	March 3, 2008

Keywords provided by National Institutes of Health Clinical Center (CC):

Skin	Complement
Humoral Immunity	Antibody
Myositis	Dermatomyositis

Additional relevant MeSH terms:

Dermatomyositis	Neuromuscular Diseases
Polymyositis	Nervous System Diseases
Myositis	

Samsung Bioepis Exhibit No. 1002
Page 1264

Muscular Diseases
Musculoskeletal Diseases
Connective Tissue Diseases
Skin Diseases

ClinicalTrials.gov processed this record on February 24, 2017

Samsung Bioepis Exhibit No. 1002
Page 1265

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (03-15)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99**)		
	Application Number	15148839
	Filing Date	2016-05-06
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN2

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Patent citation information please click the Add button. Add

U.S.PATENT APPLICATION PUBLICATIONS Remove

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20030049260	A1	2003-03-13	Bell	
	2	20040219147	A1	2004-11-04	Bell	
	3	20040038308	A1	2004-02-26	Rother et al.	
	4	20040253242	A1	2004-12-16	Bowdish et al.	
	5	20050036991	A1	2005-02-17	Fodor	

EFS Web 2.1.17

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15148839
	Filing Date	2016-05-06
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN2

	6	20050226870	A1	2005-10-13	Wang et al.	
	7	20060057679	A1	2006-03-16	O'Keefe et al.	
	8	20060263349	A1	2006-11-23	McCutcheon et al.	
	9	20160376355	A1	2016-12-29	BELL et al.	
	10	20170015741	A1	2017-01-19	BELL et al.	

If you wish to add additional U.S. Published Application citation information please click the Add button. Add

FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	2007005612	WO	A2	2007-01-11	Medimmune, Inc		
	2	2005110481	WO	A2	2005-11-24	Alexion Pharmaceuticals, Inc		
	3	2007002571	WO	A2	2007-01-04	Alexion Pharmaceuticals, Inc		

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15148839
	Filing Date	2016-05-06
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN2

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	Adis International Limited, "Eculizumab: 5G1.1, h5G1.1, Long-Acting Anti-C5 Monoclonal Antibody 5G1-1, Long-Acting Anti-C5 Monoclonal Antibody 5G1.1," Drugs in Research and Development, vol. 8 (1): 61-68 (2007).	
	2	Rother et al., "Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria," Nature Biotechnology, 25 (11) 1256-1264 (2007)	
	3	Alexion Pharma K.K. "Report on the Deliberation Results" for "Soliris for intravenous infusion 300 mg", Marh 5, 2010, pp. 1-105	
	4	Alexion Pharmaceuticals Inc., "Alexion completes enrollment in Ph II kidney disease trial," Market letter (Feb 25, 2002), 2 pages.	
	5	Alexion Pharmaceuticals Inc., "Alexion Completes Enrollment in Phase II Membranous Nephritis Clinical Trial with 5G1.1; 5G1.1 Receives Orphan Drug Designation in Membranous Nephritis," PR Newswire, February, 20, 2012, 3 pages.	
	6	Alexion Pharmaceuticals Inc., "Alexion issued C5 complement inhibitor patent for inflammatory diseases," ESPICOM Pharmaceutical and Medical Device News (Mar 19, 2002), 1 page.	
	7	Alexion Pharmaceuticals Inc., "Alexion Issued Key C5 Complement Inhibitor Patent for Inflammatory Diseases," PR Newswire(March 15, 2002) 3 pages.	
	8	Alexion Pharmaceuticals Inc., "Alexion Pharmaceuticals Reports Second Quarter and First Half Results," PR Newswire [New York] (March 8, 2001) 8 pages.	
	9	Alexion Pharmaceuticals Inc., "Alexion Pharmaceuticals Reports Second Quarter and First Half Results; - Quarter Highlighted by Successful Revision of Pexelizumab Collaboration With Procter &Gamble Pharmaceuticals and Commencement Of Phase III Pivotal PRIMO-CABG Trial," PR Newswire, March 8, 2002, 6 pages.	
	10	Alexion Pharmaceuticals Inc., "Company reports second quarter, first- half results," Biotech Week (Apr 10, 2002), 2 pages.	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15148839
	Filing Date	2016-05-06
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN2

	11	Alexion Pharmaceuticals Inc., "Eculizumab Alexion clinical data (phase III) (PNH)," R & D Focus Drug News (Feb 6, 2006), 2 pages.	
	12	Alexion Pharmaceuticals Inc., "Eculizumab Alexion clinical data (PNH)," R & D Focus Drug News (Jan 30, 2006), 2 pages.	
	13	Alexion Pharmaceuticals Inc., "Eculizumab Alexion completes enrollment for phase III PNH trial," R & D Focus Drug News, (Apr 25, 2005): 2 pages.	
	14	Alexion Pharmaceuticals Inc., "Eculizumab Alexion completes enrollment," R & D Focus Drug News (Feb 17, 2003), 2 pages.	
	15	Alexion Pharmaceuticals Inc., "Eculizumab Alexion EMEA grants request for accelerated assessment procedure," R & D Focus Drug News (Aug 28, 2006), 2 pages.	
	16	Alexion Pharmaceuticals Inc., "Eculizumab Alexion initiates second phase III study," R & D Focus Drug News, (Jan 17, 2005), 2 pages.	
	17	Alexion Pharmaceuticals Inc., "Eculizumab Alexion plans phase III trials (paroxysmal nocturnal hemoglobinuria)," R & D Focus Drug News (Aug 2, 2004), 2 pages.	
	18	Alexion Pharmaceuticals Inc., "Eculizumab Alexion Priority Review, USA (PNH)," R & D Focus Drug News (Nov 20, 2006), 2 pages.	
	19	Alexion Pharmaceuticals Inc., "Kidney Disease; Enrollment complete for phase II membranous nephritis clinical trial with 5G1.1," Drug Week, page 14 (Apr 12, 2002)	
	20	Alexion Pharmaceuticals Inc., "Kidney Disease; Enrollment complete for phase II membranous nephritis clinical trial with 5G1.1," Immunotherapy Weekly, page 18, (Apr 10, 2002)	
	21	Alexion Pharmaceuticals Inc.,"Eculizumab Alexion phase change III, USA, Europe Canada, Australia (PNH)," R & D Focus Drug News (Nov 15, 2004): 2 pages.	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15148839
	Filing Date	2016-05-06
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN2

	22	BC Cancer Agency Cancer Drug Manual, Drug Name Alemtuzumab, June 1, 2013, Pages 11.	
	23	Brekke, O. et al., "Therapeutic Antibodies For Human Diseases at the Dawn of The Twenty-First Century," Nature Reviews, Drug Discovery, vol. 2: 52-62 (2003)	
	24	ESPICOM Pharmaceutical and Medical Device News, "Eculizumab found to be well tolerated and efficacious during 52 weeks of treatment in PNH," (Jan 2, 2007), 2 pages.	
	25	European Decision Revoking The European Patent (Art. 101(2). (3)(b) EPC). 11 pages. dated July 1. 2014.	
	26	European Medicines Agency, EMA/HMPC/16633/2009 section 2, Historical Data on Medicinal Use, 2010, pages 1-40.	
	27	Fung, M. et al., "Inhibition of complement, neutrophil, and platelet activation by an anti-factor D monoclonal antibody in simulated cardiopulmonary bypass circuits," The Journal of Thoracic and Cardiovascular Surgery, vol. 122(1):113-121 (2001)	
	28	Grounds of Appeal filed on November 11,2014 in the related European Opposition, European Patent No. 1720571, pages 1-15.	
	29	Hale, et al., "Blood concentrations of alemtuzumab and antiglobulin responses in patients with chronic lymphocytic leukemia following intravenous or subcutaneous routes of administration," (2004) Blood 224(4), pages 948-955	
	30	Martin, F. et al., "Pathogenic Roles of B Cells in Human Autoimmunity: Insights from the Clinic,"Immunity, vol. 20: 517-527 (2004)	
	31	Mollnes, T. et al., "Strategies of therapeutic complement inhibition," Molecular Immunology, vol. 43: 107-121 (2006)	
	32	Mueller, J., "Humanized Porcine VCAM-Specific Monoclonal Antibodies with Chimeric IgG2/G4 Constant Regions Block Human Leukocyte Binding to Porcine Endothelial Cells," Molecular Immunology, vol. 34(6): 441-452 (1997)	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15148839
	Filing Date	2016-05-06
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN2

	33	Osborne, R., "Alexion Offers Early, Mixedphase III Data for Heart Drug," Bioworld Today, vol. 14, Issue 151 (Aug 6, 2003), 3 pages.	
	34	Osbourn, J. et al., "Current methods for the generation of human antibodies for the treatment of autoimmune diseases," Drug Discovery Today, vol. 8 (18): 845-851 (2003)	
	35	Other News To Note, BIOWORLD Today (Aug 9, 2002): 5 pages.	
	36	Pierangeli,S. et al., "Requirement of activation of complement C3 and C5 for antiphospholipid antibody-mediated thrombophilia," Arthritis & Rheumatology, vol. 52, Issue 7: 2120-2124(2005)	
	37	Ricklin, D. et al., "Complement-targeted therapeutics," Nature Biotechnology, vol. 25(11): 1265-1275 (2007)	

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature	/JAMES L ROGERS/	Date Considered	03/21/2017

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15148839
	Filing Date	2016-05-06
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN2

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Jill Gorny Sloper, Esq./	Date (YYYY-MM-DD)	2017-02-15
Name/Print	Jill Gorny Sloper, Esq.	Registration Number	60,760

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

ClinicalTrials.gov archive
A service of the U.S. National Institutes of Health — Developed by the National Library of Medicine

← History of this study ↑ Current version of this study

View of NCT00098280 on 2005_06_23

ClinicalTrials Identifier: NCT00098280
Updated: 2005_06_23

Descriptive Information

Brief title Eculizumab to Treat Paroxysmal Nocturnal Hemoglobinuria

Official title A Hemoglobin Stabilization and Transfusion Reduction Efficacy and Safety Clinical Investigation, Randomized, Multi-Center, Double-Blind, Placebo-Controlled, Using Eculizumab in Paroxysmal Nocturnal Hemoglobinuria Patients

Brief summary

This study will examine the safety and effectiveness of the experimental drug eculizumab in treating patients with paroxysmal nocturnal hemoglobinuria (PNH), a rare disorder of red blood cells that leads to premature destruction of the cells and resulting anemia. Patients may be at high risk of blood clots and may develop bone marrow failure or aplastic anemia, with low white blood cell and platelet counts. Eculizumab is a monoclonal antibody that may help improve the survival of red blood cells.

Patients 18 years of age and older with PNH who require blood transfusions for anemia and have received at least four transfusions in the 12 months preceding evaluation for this study may be eligible to enroll. Candidates are screened with a medical history, physical examination, and check of vital signs.

Participants have an electrocardiogram (EKG) and blood and urine tests, and are vaccinated against Neisseria meningitides, a common bacteria that can cause a disabling or fatal type of meningitis. They then enter an observation phase of the study, with monthly visits during which they complete a questionnaire; update their health status, transfusion record, and medication use; have their vital signs checked and PNH symptoms evaluated; have blood and urine tests; and receive a transfusion, if necessary. These visits continue for up to 3 months until patients receive a "qualifying" transfusion; that is, a transfusion given as a consequence of a certain hemoglobin level with symptoms or a different level without symptoms.

Patients are then randomly assigned to receive either eculizumab or a placebo (salt solution with no active ingredient). Both study medications are given intravenously (through a vein) over 30 minutes once a week for five doses and then once every 2 weeks for another 11 doses. At each treatment visit (study weeks 0-24), patients update their health status, transfusion records, and medication use; have their vital signs checked; and provide a blood sample. At various visits, they also complete a questionnaire, provide a urine sample and have an EKG. At the last treatment visit (week 26 or the final visit for patients who end their participation before visit 18) patients have a complete physical examination in addition to the procedures listed above.

Detailed description

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal disorder of the hematopoietic stem cell characterized by intravascular hemolysis, hemoglobinuria,

anemia, and thrombosis. The clinical features of PNH result from the lack of one or more of the GPI-linked proteins that serve to protect cells from autologous complement mediated attack. Two such proteins, CD55 (decay accelerating factor) and CD59 (reactive lysis inhibitor) have been shown to be absent from PNH erythrocytes and platelets as well as other cell types.

Evidence strongly suggests that lack of the terminal complement inhibitor CD59 is responsible for the sensitivity of PNH erythrocytes and platelets to the effects of autologous complement. Since the pathogenesis of PNH is due to the inability of PNH red cells and platelets to inhibit the activation of terminal complement, it is logical to hypothesize that a terminal complement inhibitor could effectively stop the intravascular hemolysis, obviate or lessen the need for transfusions, and possibly decrease the propensity of life threatening thrombosis. Eculizumab is a humanized monoclonal antibody that like CD59 inhibits terminal complement.

This study is a randomized, double-blind, placebo controlled, multi-center study of eculizumab or placebo administered intravenously to approximately 75 PNH patients. The study is designed to evaluate the safety of eculizumab in transfusion dependent patients with paroxysmal nocturnal hemoglobinuria (PNH) and to determine if the administration of this terminal complement inhibitor could provide a safe and effective substitute for CD59 function.

Phase	III
Study type	Interventional
Condition	Hemoglobinuria, Paroxysmal
Intervention	Drug: Eculizumab
Reference	Citation: Hillmen P, et al. Natural history of paroxysmal nocturnal hemoglobinuria. N Engl J Med. 1995 Nov 9;333(19):1253-8. MEDLINE: 7566002
Reference	Citation: Tabbara IA. Hemolytic anemias. Diagnosis and management. Med Clin North Am. 1992 May;76(3):649-68. Review. MEDLINE: 1578962
Reference	Citation: Rosse WF. Phosphatidylinositol-linked proteins and paroxysmal nocturnal hemoglobinuria. Blood. 1990 Apr 15;75(8):1595-601. Review. No abstract available. MEDLINE: 2183885
URL	http://clinicalstudies.info.nih.gov/detail/A_2005-H-0048.html
See also	Detailed Web Page

Recruitment Information

Status	Recruiting
Start date	2004-12-02

Criteria

INCLUSION CRITERIA - Screening Period:

Individuals at least 18 years of age;

Patients must have required at least 4 episodes of transfusions in the 12 months prior to Visit 1 for anemia or anemia-related symptoms;

Presence of a GPI deficient red blood cell clone (type II cells) by flow cytometry of greater than or equal to 10%;

Samsung Bioepis Exhibit No. 1002
Page 1275

Patients taking erythropoietin must be on a stable dose for 26 weeks prior to visit 1 and the dose must be expected to remain stable during the Observation Period and the Treatment Phase;

Patients taking immunosuppressants must be on a stable dose for at least 26 weeks prior to Visit 1 and the dose must be expected to remain stable during the Observation period and the Treatment Phase;

Patients taking corticosteroids must be on a stable dose for at least 4 weeks prior to Visit 1. The dose level may be decreased during the study as clinically appropriate. If the corticosteroids need to be increased, they may not be increased above the dose reported at Visit 1;

Patients are allowed to take coumadin, but must be at a stable international normalized ratio (INR) level for 4 weeks prior to Visit 1 and are expected to maintain a stable INR level during the Observation Period and the Treatment Phase;

Patients taking iron supplements or folic acid must be on a stable dose for 4 weeks prior to Visit 3 and be expected to remain stable during Observation Period and the Treatment Phase;

Patients are allowed to take low molecular weight heparin, but must be on a stable dose for 4 weeks prior to Visit 1 and the dose must be expected to remain stable during Observation period and the Treatment Phase;

Patient must be willing and able to give written informed consent;

Patients must be vaccinated against N. meningitidis at Visit 1 or at least 14 days prior to Visit 3;

Patient must avoid conception during the trial using a method that is most appropriate for their physical state and culture.

INCLUSION CRITERIA - Observation Period:

Documented LDH level greater than or equal to 1.5 x upper limit of normal (ULN) either at Visit 1 or during the Observation Period;

Patients who have received one PRBC transfusion during the Observation Period at a hemoglobin value of less than or equal to 9 gm/dL with symptoms or at a hemoglobin value of less than or equal to 7 gm/dL without symptoms, and also within 1.5 gm/dL of the mean hemoglobin pre-transfusion value for the previous 12 months. This transfusion should be in accordance with that patient's individual transfusion hemoglobin algorithm, and occur within 48 hours of the hemoglobin sample that precipitated the transfusion;

Platelet count greater than or equal to 100,000/mm(3) either at Visit 1 or during the Observation Period.

EXCLUSION CRITERIA:

Patients whose mean hemoglobin levels prior to transfusion over the previous 12 months is greater than 10.5 gm/dL;

Absolute Neutrophil count less than or equal to 500/microL;

Samsung Bioepis Exhibit No. 1002
Page 1276

Presence or suspicion of active bacterial infection, in the opinion of the Investigator, at Visit 3 or recurrent bacterial infections;

Known or suspected hereditary complement deficiency;

Participation in any other investigational drug trial or exposure to other investigational agent, device, or procedure within 30 days prior to Visit 1;

Pregnant, breast-feeding, or intending to conceive during the course of the study, including the Post-treatment Phase;

History of meningococcal disease;

History of bone marrow transplantation;

Any condition that, in the opinion of the Investigator, could increase the patient's risk by participating in the study or confound the outcome of the study.

Gender	Both
Healthy volunteers	Yes
Expected enrollment	75

Administrative Data

Secondary ID	05-H-0048
Sponsor	National Heart, Lung and Blood Institute (NHLBI)

Samsung Bioepis Exhibit No. 1002
Page 1277

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	180	(leonard near3 bell).in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/03/02 09:55
L2	399	(russell near3 rother).in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/03/02 09:55
L3	188	(alexion near3 pharmaceuticals).as.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/03/02 09:56
L4	200	(eculizumab).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/03/02 09:56
L5	292	((paroxysmal near3 nocturnal) or PNH).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/03/02 09:56
L6	22	L4 and L5	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/03/02 09:56
L7	6	L1 and L6	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/03/02 09:56
L8	4	L2 and L6	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/03/02 10:00
L9	5	L3 and L6	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2017/03/02 10:02

EAST Search History (Interference)

<This search history is empty>

3/2/2017 10:08:01 AM

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 November 2005 (24.11.2005)

PCT

(10) International Publication Number
WO 2005/110481 A2

(51) International Patent Classification[7]: A61K 45/06

(21) International Application Number:
PCT/US2005/017048

(22) International Filing Date: 16 May 2005 (16.05.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/571,444 14 May 2004 (14.05.2004) US

(71) Applicants *(for all designated States except US)*: **ALEXION PHARMACEUTICALS, INC.** [US/US]; 352 Knotter Drive, Cheshire, CT 06410 (US). **THE UNIVERSITY OF WESTERN ONTARIO** [CA/CA]; 1151 Richmond Street North, Stevenson-Lawson Building, Room 328, London, Ontario N6A 5B8 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ROTHER, Russell, P.** [US/US]; 25 Blue Trail Drive, Prospect, CT 06712 (US). **WANG, Hao** [CA/CA]; 480 Sandybrook Drive, London, Ontario N5X 4H2 (CA). **ZHONG, Zhen** [CA/CA]; 60 Nathaniel Court, London, Ontario N5X 2N5 (CA).

(74) Agents: **WU, Xuqiong** et al.; Fish & Neave IP Group of, Ropes & Gray LLP, One International Place, Boston, MA 02110-2624 (US).

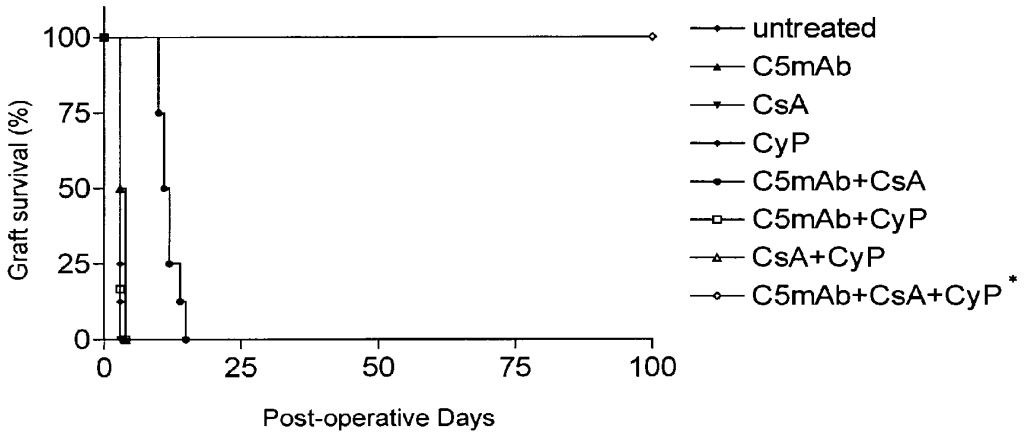
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PROLONGATION OF SURVIVAL OF AN ALLOGRAFT BY INHIBITING COMPLEMENT ACTIVITY



(57) Abstract: Methods of prolonging survival of allotransplanted cells, tissues or organs are presented. These methods are directed to administering to the allotransplant recipient an inhibitor of complement activity together with one or more immunosuppressants. The inhibitor of complement activity is administered chronically. These methods have been determined to aid in preventing chronic rejection of allografts. These methods can additionally be used in cases in which the recipient has been presensitized to the allograft or in which there is an ABO mismatch between the allograft and the recipient.

WO 2005/110481 A2

PROLONGATION OF SURVIVAL OF AN ALLOGRAFT BY INHIBITING COMPLEMENT ACTIVITY

RELATED APPLICATIONS

This application claims the benefit of and priority to U.S. Provisional Patent Application No. 60/571,444, filed May 14, 2004, the disclosure of which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

The present disclosure relates to methods for prolonging survival of an allograft in a mammal. In particular, the present disclosure relates to prolonging survival of an allograft by administering an inhibitor of complement or terminal complement formation, especially an inhibitor of complement C5 cleavage, in addition to one or more drugs that are immunosuppressant.

BACKGROUND

Organ transplantation is the preferred treatment for most patients with chronic organ failure. Although transplantation of kidney, liver, lung, and heart offers an excellent opportunity for rehabilitation as recipients return to a more normal lifestyle, it is limited by the medical/surgical suitability of potential recipients, an increasing shortage of donors, and premature failure of transplanted organ function.

Transplantation of cells, tissues and organs has become very common and is often a life-saving procedure. Organ transplantation is the preferred treatment for most patients with chronic organ failure. Despite great improvement in treatments to inhibit rejection, rejection continues to be the single largest impediment to successful organ transplantation. Rejection includes not only acute rejection but also chronic rejection. One-year survival rates for transplanted kidneys average 88.3% with kidneys from deceased donors and 94.4% with kidneys received from living donors. The corresponding five year survival rates for the transplanted kidneys are 63.3% and 76.5% (OPTN/SRTR Annual Report, 2002). For livers the one year survival rates are 80.2% and 76.5% for livers from deceased and living donors, respectively. The corresponding five year liver graft survival rates are 63.5% and 73.0% (OPTN/SRTR Annual Report, 2002). The use of immunosuppressant drugs, especially cyclosporin A and more recently tacrolimus, has dramatically improved the success rate of organ transplantation especially by preventing acute rejection. But as the numbers above

Samsung Bioepis Exhibit No. 1002
Page 1280

show, there is still a need to improve the success rates, both short-term and especially long-term. As seen from the above numbers for kidney and liver transplants, the five year failure rates for these transplanted organs are on the order of 25-35%. In the year 2001 alone there were more than 23,000 patients who received an organ transplant of which approximately 19,000 were kidney or liver (OPTN/SRTR Annual Report, 2002). For this one year of transplants alone, with present techniques it can be expected that approximately 5,000-6,000 of these transplanted kidneys and livers will fail within 5 years. These numbers do not even include other transplanted organs or transplanted tissues or cells such as bone marrow.

There are multiple types of transplants. These are described in Abbas et al., 2000. A graft transplanted from one individual to the same individual is called an autologous graft or autograft. A graft transplanted between two genetically identical or syngeneic individual is called a syngeneic graft. A graft transplanted between two genetically different individuals of the same species is called an allogeneic graft or allograft. A graft transplanted between individuals of different species is called a xenogeneic graft or xenograft. The molecules that are recognized as foreign on allografts are called alloantigens and those on xenografts are called xenoantigens. The lymphocytes or antibodies that react with alloantigens or xenoantigens are described as being alloreactive or xenoreactive, respectively.

Currently more than 40,000 kidney, heart, lung, liver and pancreas transplants are performed in the United States each year (Abbas et al., 2000). Other possible transplants include, but are not limited to, vascular tissue, eye, cornea, lens, skin, bone marrow, muscle, connective tissue, gastrointestinal tissue, nervous tissue, bone, stem cells, islets, cartilage, hepatocytes, and hematopoietic cells. Unfortunately, there are many more candidates for a transplant than there are donors. To overcome this shortage, a major effort is being made to learn how to use xenografts. While progress is being made in this field, the fact is that at present most transplants are allografts. An allogeneic transplant, while presently being more likely to be successful than a xenogeneic transplant, must surmount numerous obstacles to be successful. There are several types of immunological attacks made by the recipient against the donor organ which can lead to rejection of the allograft. These include hyperacute rejection, acute vascular rejection (including accelerated humoral rejection and de novo acute humoral rejection), and chronic rejection. Rejection is normally a result of T-cell mediated or humoral antibody attack, but may include additional secondary factors such as the effects of complement and cytokines.

Samsung Bioepis Exhibit No. 1002
Page 1281

An ever growing gap between the number of patients requiring organ transplantation and the number of donor organs available has become a major problem throughout the world. Park et al., 2003. Individuals who have developed anti-HLA antibodies are said to be immunized or sensitized. Gloor, 2005. HLA sensitization is the major barrier to optimal utilization of organs from living donors in clinical transplantation (Warren et al., 2004) due to the development of severe antibody-mediated rejection (ABMR). For example, more than 50% of all individuals awaiting kidney transplantation are presensitized patients (Glotz et al., 2002) who have elevated levels of broadly reactive alloantibodies, resulting from multiple transfusions, prior failed allografts, or pregnancy (Kupiec-Weglinski, 1996). The role of ABMR is currently one of the most dynamic areas of study in transplantation, due to recognition that this type of rejection can lead to either acute or chronic loss of allograft function. Mehra et al., 2003. Numerous cases of ABMR, including hyperacute rejection (HAR) or accelerated humoral rejection (ACHR), have been reported that are characterized by acute allograft injury that is resistant to potent anti-T cell therapy, the detection of circulating donor specific antibodies, and the deposition of complement components in the graft. ABMR with elevated circulating alloantibodies and complement activation that occurs in 20-30% of acute rejection cases has a poorer prognosis than cellular rejection. Mauiyyedi et al., 2002.

Highly presensitized patients, who exhibit high levels of alloantibodies, usually suffer an immediate and aggressive HAR. In clinical practice, with great efforts and significant advances in technology, HAR is avoided by obtaining a pretransplant lymphocytotoxic cross-match to identify sensitized patients with antibodies specific for donor HLA antigens. However, circulating antibodies against donor HLA or other non-MHC endothelial antigens may also be responsible for a delayed form of acute humoral rejection, which is associated with an increased incidence of graft loss. Collins et al., 1999. Therefore, development of a novel presensitized animal model to mimic ABMR in clinical settings would be beneficial to studies on the mechanism, and to the much needed progress in the management of allograft rejection in presensitized hosts.

Some highly presensitized patients can benefit from intervention programs such as immunoadsorption (Palmer et al.,1989; Ross et al., 1993; Kriaa et al., 1995), plasmapheresis and intravenous immunoglobulin (Sonnenday et al., 2002; Rocha et al., 2003), that have been designed and implemented to temporarily eliminate anti-donor antibodies. However, in addition to their benefits, the aforementioned therapies carry with them numerous drawbacks

Samsung Bioepis Exhibit No. 1002
Page 1282

as some individuals are less susceptible to their effects (Kriaa et al., 1995; Hakim et al., 1990; Glotz et al., 1993; Tyan et al., 1994); they are extremely expensive, time-consuming, and risky (Salama et al., 2001). Moreover, the transient and variable effect of these protocols has limited their impact. Glotz et al., 2002; Kupin et al., 1991; Schweitzer et al., 2000. Therefore, developing novel strategies to reduce the risk and cost in prevention of ABMR would be beneficial to presensitized recipients receiving an allograft.

SUMMARY

Accordingly, methods of prolonging survival of transplanted cells, tissues or organs are provided. In particular, methods of prolonging survival of allotransplanted cells, tissues or organs are provided. These methods are directed to using one or more immunosuppressants in addition to an inhibitor of complement activity. Use of one or more immunosuppressants and an inhibitor of complement activity in the manufacture of one or more medicaments or medicament packages is also provided. Such medicaments or medicament packages are useful in prolonging survival of an allograft in a subject mammal.

In certain embodiments, the inhibition of complement activity is effected by chronic administration of a drug directed against complement C5. A preferred drug that inhibits complement activity is an antibody specific to one or more components of complement, for example, C5. In certain preferred embodiments, the antibody inhibits the cleavage of C5 and thereby inhibits the formation of both C5a and C5b-9. The antibody may be, e.g., a monoclonal antibody, a chimeric antibody (e.g., a humanized antibody), an antibody fragment (e.g., Fab), a single chain antibody, an Fv, or a domain antibody. The recipient is also treated with one or more immunosuppressive drugs, for example, cyclosporin A.

In certain embodiments, either an MHC mismatched recipient (i.e., a mammalian recipient of an MHC mismatched allograft), a presensitized recipient or an ABO mismatched recipient (i.e., a mammalian recipient of an AMB mismatched allograft) is treated. In this model, the recipient is again chronically treated with a complement inhibitor, preferably an anti-C5 monoclonal antibody, together with immunosuppressive drugs, preferably a chronic administration of cyclosporin A and a short-term administration of cyclophosphamide. This triple therapy results in extended graft survival in the presensitized allotransplant recipient.

The present disclosure also provides methods of prolonging survival of an allograft in a mammalian recipient by administering to the recipient agents that modulate the level and/or ratio of subclasses and/or isotypes of anti-donor immunoglobulins (Ig) in the recipient. In

Samsung Bioepis Exhibit No. 1002
Page 1283

certain embodiments, an agent that reduces the level of anti-donor IgG1 in the recipient is preferred. In certain embodiments, an agent that increases the level of anti-donor IgG2a and/or IgG2b in the recipient is preferred. In certain embodiments, an agent that reduces the ratio of anti-donor IgG1/anti-donor IgG2a or IgG2b in the recipient is preferred.

The present disclosure also provides a method of prolonging survival of an allograft in a second mammalian recipient using an allograft that has been accommodated in a first mammalian recipient (i.e., the allograft has prolonged survival in the first recipient). The present disclosure further provides an allograft that is resistant to anti-donor antibodies in a mammalian recipient, and the allograft is prepared from a first recipient that has accommodated the allograft. In preferred embodiments, the first recipient has accommodated the allograft by receiving a treatment as described herein, such a triple therapy treatment involving administering to the first recipient a drug that inhibits complement activity and two immunosuppressive agents.

Further provided are pharmaceutical packages. A pharmaceutical package of the present disclosure may comprise a drug that inhibits complement activity and at least one immunosuppressive agent. The pharmaceutical package may further comprise a label for chronic administration. The pharmaceutical package may also comprise a label for self-administration by a patient, for example, a recipient of a transplant graft, or instructions for a caretaker of a recipient of a transplant graft. In certain embodiments, the drug and the agent in the pharmaceutical package are in a formulation or separate formulations that are suitable for chronic administration and/or self-administration.

The present disclosure also provides lyophilized formulations and formulations suitable for injection. Certain embodiments provide a lyophilized antibody formulation comprising an antibody that inhibits complement activity and a lyoprotectant. In preferred embodiments, the antibody formulation is suitable for chronic administration, for example, the antibody formulation is stable. Alternative embodiments provide an injection system comprising a syringe; the syringe comprises a cartridge containing an antibody that inhibits complement activity and is in a formulation suitable for injection.

An antibody employed in various embodiments of the present disclosure preferably inhibits the formation of terminal complement or C5a. In certain embodiments, antibody inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment. The whole antibody or antibody fragment may be a human, humanized,

Samsung Bioepis Exhibit No. 1002
Page 1284

chimerized or deimmunized antibody or antibody fragment. In certain embodiments, the whole antibody or antibody fragment may inhibit cleavage of complement C5. In certain embodiments, the antibody fragment is a Fab, an F(ab')2, an Fv, a domain antibody, or a single-chain antibody. In preferred embodiments, the antibody fragment is pexelizumab. In alternative preferred embodiments, the whole antibody is eculizumab.

In certain embodiments, a drug, such as an antibody, that inhibits complement activity is present in unit dosage form, which can be particularly suitable for self-administration. Similarly, an immunosuppressive agent of the present disclosure may also be present in unit dosage form.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D show anti-donor antibody levels in presensitized versus unsensitized recipients under different treatments.

Figures 2A and 2B show comparison between triple therapy using anti-C5 antibody, CsA and CyP in presensitized allograft recipients and combination therapy using only anti-C5 antibody and CsA in presensitized allograft recipients. Figure 2A compares heart-allograft survival in various recipients under different treatments as indicated. Figure 2B shows histology and immunohistology, for example, for lymphocyte infiltration in heart allografts of recipients in different groups.

Figure 3 shows blocked terminal complement activity by anti-C5 antibody as compared to immunosuppressive agents.

Figures 4A-4D compare levels of anti-donor antibodies in presensitized recipients of allografts under monotherapy with anti-C5 antibody alone, double combination therapy with anti-C5 antibody and CsA, and triple combination therapy with anti-C5 antibody, CsA and CyP.

Figures 5A and 5B show change of ratios of IgG isotypes in allograft recipients that were untreated or under different treatments.

Figure 6 shows high-level expression of Bcl-2 and Bcl-xl proteins in long-term surviving heart grafts as compared to heart grafts of untreated animals.

Figure 7 shows improved second transplantation (re-transplantation) of an accommodated graft from a first transplantation recipient.

Figure 8 shows results from re-transplantation experiments.

Samsung Bioepis Exhibit No. 1002
Page 1285

Figure 9 shows results from re-transplantation experiments.

DETAILED DESCRIPTION

Overview: Rejection of Transplants or Grafts

Hyperacute rejection occurs within minutes to hours after transplant and is due to preformed antibodies to the transplanted tissue antigens. It is characterized by hemorrhage and thrombotic occlusion of the graft vasculature. The binding of antibody to endothelium activates complement, and antibody and complement induce a number of changes in the graft endothelium that promote intravascular thrombosis and lead to vascular occlusion, the result being that the grafted organ suffers irreversible ischemic damage (Abbas et al., 2000). Hyperacute rejection is often mediated by preexisting IgM alloantibodies, e.g., those directed against the ABO blood group antigens expressed on red blood cells. This type of rejection, mediated by natural antibodies, is the main reason for rejection of xenotransplants. Hyperacute rejection due to natural IgM antibodies is no longer a major problem with allografts because allografts are usually selected to match the donor and recipient ABO type. Hyperacute rejection of an ABO matched allograft may still occur, usually mediated by IgG antibodies directed against protein alloantigens, such as foreign MHC molecules, or against less well defined alloantigens expressed on vascular endothelial cells. Such antibodies may arise as a result of prior exposure to alloantigens through blood transfusion, prior transplantation, or multiple pregnancies (this prior exposure being referred to as "presensitization"). Abbas et al., 2000.

Acute rejection is a process of vascular and parenchymal injury mediated by T cells, macrophages, and antibodies that usually begins after the first week of transplantation. Abbas et al., 2001. T lymphocytes play a central role in acute rejection by responding to alloantigens, including MHC molecules, present on vascular endothelial and parenchymal cells. The activated T cells cause direct lysis of graft cells or produce cytokines that recruit and activate inflammatory cells, which cause necrosis. Both $CD4^+$ and $CD8^+$ cells may contribute to acute rejection. The destruction of allogeneic cells in a graft is highly specific and a hallmark of $CD8^+$ cytotoxic T lymphocyte killing. Abbas et al., 2000. $CD4^+$ T cells may be important in mediating acute graft rejection by secreting cytokines and inducing delayed-type hypersensitivity-like reactions in grafts, with some evidence available that indicates that $CD4^+$ T cells are sufficient to mediate acute rejection. Abbas et al., 2000. Antibodies can also mediate acute rejection after a graft recipient mounts a humoral immune

Samsung Bioepis Exhibit No. 1002
Page 1286

response to vessel wall antigens and the antibodies that are produced bind to the vessel wall and activate complement. Abbas et al., 2000.

Chronic rejection is characterized by fibrosis with loss of normal organ structures occurring over a prolonged period. The pathogenesis of chronic rejection is less well understood than that of acute rejection. Graft arterial occlusion may occur as a result of the proliferation of intimal smooth muscle cells (Abbas et al., 2000). This process is called accelerated or graft arteriosclerosis and can develop in any vascularized organ transplant within 6 months to a year after transplantation.

For a transplant to be successful, the several modes of rejection must be overcome. Multiple approaches are utilized in preventing rejection. This may require administration of immunosuppressants, often several types to prevent the various modes of attack, e.g., inhibition of T-cell attack, antibodies, and cytokine and complement effects. Prescreening of donors to match them with recipients is also a major factor in preventing rejection, especially in preventing hyperacute rejection. Immunoadsorption of anti-HLA antibodies prior to grafting may reduce hyperacute rejection. Prior to transplantation the recipient or host may be administered anti-T cell reagents, e.g., the monoclonal antibody OKT3, Anti-Thymocyte Globulin (ATG), cyclosporin A, or tacrolimus (FK 506). Additionally, glucocorticoids and/or azathioprine may be administered to the host prior to transplant. Drugs used to aid in preventing transplant rejection include, but are not limited to, ATG or ALG, OKT3, daclizumab, basiliximab, corticosteroids, 15-deoxyspergualin, cyclosporins, tacrolimus, azathioprine, methotrexate, mycophenolate mofetil, 6-mercaptopurine, bredinin, brequinar, leflunamide, cyclophosphamide, sirolimus, anti-CD4 monoclonal antibodies, CTLA4-Ig, anti-CD154 monoclonal antibodies, anti-LFA1 monoclonal antibodies, anti-LFA-3 monoclonal antibodies, anti-CD2 monoclonal antibodies, and anti-CD45.

Allografts are rejected in part by the activation of T cells. The transplant recipient mounts a rejection response following $CD4^+$ T cell recognition of foreign antigens in the allograft. These antigens are encoded by the major histocompatibility complex (MHC). There are both Class I and Class II MHC molecules. In humans the class I MHC molecules are HLA-A, HLA-B, and HLA-C. The class II MHC molecules in humans are called HLA-DR, HLA-DQ and HLA-DP. In mice the class I MHC molecules are H-2K, H-2D and H-2L and the class II MHC molecules are I-A and I-E. When $CD4^+$ T cells bind the foreign MHC antigens they are activated and undergo clonal proliferation. The activated T cells secrete cytokines which aid in activating monocytes/macrophages, B cells and cytotoxic $CD8^+$ T

Samsung Bioepis Exhibit No. 1002
Page 1287

cells. The activated monocytes/macrophages release agents which result in tissue damage, the B cells produce alloantibodies which lead to complement mediated tissue destruction, and the $CD8^+$ T cells kill graft cells in an antigen-specific manner through induction of apoptosis and cell lysis.

Immunosuppressive Agents

The numerous drugs utilized to delay graft rejection (i.e., to prolong their survival) work in a variety of ways. Immunosuppressive agents are widely used. See Stepkowski, 2000, for a review of the mechanism of action of several immunosuppressive drugs. Cyclosporin A is one of the most widely used immunosuppressive drugs for inhibiting graft rejection. It is an inhibitor of interleukin-2 or IL-2 (it prevents mRNA transcription of interleukin-2). More directly, cyclosporin inhibits calcineurin activation that normally occurs upon T cell receptor stimulation. Calcineurin dephosphorylates NFAT (nuclear factor of activated T cells) enabling it to enter the nucleus and bind to interleukin-2 promoter. By blocking this process, cyclosporin A inhibits the activation of the $CD4^+$ T cells and the resulting cascade of events which would otherwise occur. Tacrolimus is another immunosuppressant that acts by inhibiting the production of interleukin-2.

Rapamycin (Sirolimus), SDZ RAD, and interleukin-2 receptor blockers are drugs that inhibit the action of interleukin-2 and therefore prevent the cascade of events described above.

Inhibitors of purine or pyrimidine biosynthesis are also used to inhibit graft rejection. These prevent DNA synthesis and thereby inhibit cell division including the ability of T cells to divide. The result is the inhibition of T cell activity by preventing the formation of new T cells. Inhibitors of purine synthesis include azathioprine, methotrexate, mycophenolate mofetil (MMF) and mizoribine (bredinin). Inhibitors of pyrimidine synthesis include brequinar sodium and leflunomide. Cyclophosphamide is an inhibitor of both purine and pyrimidine synthesis.

Yet another method for inhibiting T cell activation is to treat the recipient with antibodies to T cells. OKT3 is a murine monoclonal antibody against CD3 which is part of the T cell receptor. This antibody inhibits the T cell receptor and suppresses T cell activation.

Numerous other drugs and methods for delaying allotransplant rejection are known to and used by those of skill in the art. One approach has been to deplete T cells, e.g., by irradiation. This has often been used in bone marrow transplants, especially if there is a

Samsung Bioepis Exhibit No. 1002
Page 1288

partial mismatch of major HLA. Administration to the recipient of an inhibitor (blocker) of the CD40 ligand-CD40 interaction and/or a blocker of the CD28-B7 interaction has been used (U.S. Patent 6,280,957). Published PCT patent application WO 01/37860 teaches the administration of an anti-CD3 monoclonal antibody and IL-5 to inhibit the Th1 immune response. Published PCT patent application WO 00/27421 teaches a method for prophylaxis or treatment of corneal transplant rejection by administering a tumor necrosis factor-α antagonist. Glotz et al. (2002) show that administration of intravenous immunoglobulins (IVIg) can induce a profound and sustained decrease in the titers of anti-HLA antibodies thereby allowing a transplant of an HLA-mismatched organ. Similar protocols have included plasma exchanges (Taube et al., 1984) or immunoadsorption techniques coupled to immunosuppressive agents (Hiesse et al., 1992) or a combination of these (Montgomery et al., 2000). Changelian et al. (2003) teach a model in which immunosuppression is caused by an oral inhibitor of Janus kinase 3 (JAK3) which is an enzyme necessary for the proper signaling of cytokine receptors which use the common gamma chain (γc) (Interleukins-2, -4, -7, -9, -15, -21), the result being an inhibition of T cell activation. Antisense nucleic acids against ICAM-1 have been used alone or in combination with a monoclonal antibody specific for leukocyte-function associated antigen 1 (LFA-1) in a study of heart allograft transplantation (Stepkowski, 2000). Similarly, an anti-ICAM-1 antibody has been used in combination with anti-LFA-1 antibody to treat heart allografts (Stepkowski, 2000). Antisense oligonucleotides have additionally been used in conjunction with cyclosporin in rat heart or kidney allograft models, resulting in a synergistic effect to prolong the survival of the grafts (Stepkowski, 2000). Chronic transplant rejection has been treated by administering an antagonist of TGF-β which is a cytokine involved in differentiation, proliferation and apoptosis (U.S. Patent Application Publication US 2003/0180301).

Complement and Transplant/Graft Rejection

The role of complement in transplant rejection is well known. This is especially true in the case of xenotransplantation, but complement also plays a role in allotransplant rejection. For review, see Platt and Saadi, 1999. One aspect of complement's role is that ischemia-reperfusion injury may occur at the time that an organ graft is reperfused with the blood of the recipient. Complement may also cause some manifestations of allograft rejection.

The complement system is described in detail in U.S. Patent 6,355,245. The complement system acts in conjunction with other immunological systems of the body to

Samsung Bioepis Exhibit No. 1002
Page 1289

defend against intrusion of cellular and viral pathogens. There are at least 25 complement proteins, which are found as a complex collection of plasma proteins and membrane cofactors. The plasma proteins make up about 10% of the globulins in vertebrate serum. Complement components achieve their immune defensive functions by interacting in a series of intricate but precise enzymatic cleavage and membrane binding events. The resulting complement cascade leads to the production of products with opsonic, immunoregulatory, and lytic functions.

The complement cascade progresses via the classical pathway or the alternative pathway. These pathways share many components and, while they differ in their initial steps, they converge and share the same "terminal complement" components (C5 through C9) responsible for the activation and destruction of target cells.

The classical complement pathway is typically initiated by antibody recognition of and binding to an antigenic site on a target cell. The alternative pathway is usually antibody independent and can be initiated by certain molecules on pathogen surfaces. Both pathways converge at the point where complement component C3 is cleaved by an active protease (which is different in each pathway) to yield C3a and C3b. Other pathways activating complement attack can act later in the sequence of events leading to various aspects of complement function.

C3a is an anaphylatoxin. C3b binds to bacterial and other cells, as well as to certain viruses and immune complexes, and tags them for removal from the circulation. C3b in this role is known as opsonin. The opsonic function of C3b is considered to be the most important anti-infective action of the complement system. Patients with genetic lesions that block C3b function are prone to infection by a broad variety of pathogenic organisms, while patients with lesions later in the complement cascade sequence, i.e., patients with lesions that block C5 functions, are found to be more prone only to Neisseria infection, and then only somewhat more prone (Fearon, 1983).

C3b also forms a complex with other components unique to each pathway to form classical or alternative C5 convertase, which cleaves C5 into C5a and C5b. C3 is thus regarded as the central protein in the complement reaction sequence since it is essential to both the alternative and classical pathways (Wurzner et al., 1991). This property of C3b is regulated by the serum protease Factor I, which acts on C3b to produce iC3b. While still functional as opsonin, iC3b cannot form an active C5 convertase.

Samsung Bioepis Exhibit No. 1002
Page 1290

C5 is a 190 kDa beta globulin found in normal serum at approximately 75 μg/mL (0.4 μM). C5 is glycosylated, with about 1.5-3 percent of its mass attributed to carbohydrate. Mature C5 is a heterodimer of a 999 amino acid 115 kDa alpha chain that is disulfide linked to a 656 amino acid 75 kDa beta chain. C5 is synthesized as a single chain precursor protein product of a single copy gene (Haviland et al., 1991). The cDNA sequence of the transcript of this gene predicts a secreted pro-C5 precursor of 1659 amino acids along with an 18 amino acid leader sequence.

The pro-C5 precursor is cleaved after amino acid 655 and 659, to yield the beta chain as an amino terminal fragment (amino acid residues +1 to 655) and the alpha chain as a carboxyl terminal fragment (amino acid residues 660 to 1658), with four amino acids deleted between the two.

C5a is cleaved from the alpha chain of C5 by either alternative or classical C5 convertase as an amino terminal fragment comprising the first 74 amino acids of the alpha chain (i.e., amino acid residues 660-733). Approximately 20 percent of the 11 kDa mass of C5a is attributed to carbohydrate. The cleavage site for convertase action is at or immediately adjacent to amino acid residue 733. A compound that would bind at or adjacent to this cleavage site would have the potential to block access of the C5 convertase enzymes to the cleavage site and thereby act as a complement inhibitor.

C5 can also be activated by means other than C5 convertase activity. Limited trypsin digestion (Minta and Man, 1977; Wetsel and Kolb, 1982) and acid treatment (Yamamoto and Gewurz, 1978; Vogt et al., 1989) can also cleave C5 and produce active C5b.

C5a is another anaphylatoxin. C5b combines with C6, C7, and C8 to form the C5b-8 complex at the surface of the target cell. Upon binding of several C9 molecules, the membrane attack complex (MAC, C5b-9, terminal complement complex-TCC) is formed. When sufficient numbers of MACs insert into target cell membranes the openings they create (MAC pores) mediate rapid osmotic lysis of the target cells. Lower, non-lytic concentrations of MACs can produce other effects. In particular, membrane insertion of small numbers of the C5b-9 complexes into endothelial cells and platelets can cause deleterious cell activation. In some cases activation may precede cell lysis.

As mentioned above, C3a and C5a are anaphylatoxins. These activated complement components can trigger mast cell degranulation, which releases histamine and other mediators of inflammation, resulting in smooth muscle contraction, increased vascular

Samsung Bioepis Exhibit No. 1002
Page 1291

permeability, leukocyte activation, and other inflammatory phenomena including cellular proliferation resulting in hypercellularity. C5a also functions as a chemotactic peptide that serves to attract pro-inflammatory granulocytes to the site of complement activation.

Complement-binding recipient antibodies to donor alloantigens are considered to be the main cause of hyperacute graft rejection. Owing to pretransplant crossmatch testing, this prototype of humoral rejection is now rarely observed (Regele et al., 2001). Data are now showing that humoral immune mechanisms might contribute to other types of allograft rejection (Regele et al., 2001). High levels of panel reactive antibodies indicating humoral presensitization were found to be associated with inferior kidney graft survival (Opelz, 1992), the appearance of alloantibodies during the post-transplant period has been reported to predict poor graft outcome (Jeannet et al., 1970; Halloran et al., 1992), and selective removal of recipient IgG by immunoadsorption reversed some rejection episodes indicating the contribution of humoral immune mechanisms to rejection (Persson et al., 1995; Böhmig et al., 2000). Complement activation within a graft might indicate antibody-mediated graft injury. The complement cleavage product C4d is a marker for activation of the antibody-dependent classical pathway. Capillary C4d deposits in kidney allograft biopsies were associated with poor graft outcome.

Recently increasing evidence indicates that complement activation significantly contributes to the sensitization of allograft recipients and the development of tissue injury in allografts (Platt et al., 1999). Antibodies are the most thoroughly investigated mediators of activating the classical complement pathway. Clinically, alloantibodies are known to activate complement (Baldwin et al., 2001). Halloran and Collins indicate that C4d deposition in peritubular capillaries of renal allografts is a sensitive and diagnostic marker of acute humoral rejection that correlates strongly with the presence of circulating donor-specific antibodies (Collins et al., 1999; Halloran, 2003). Further supporting evidence is seen in animals with complement inhibition (Pratt et al., 1996; Pruitt et al., 1991; Forbes et al., 1978) or deficiency (Pratt et al., 2000; Baurer et al., 1995) which exhibit significantly reduced inflammatory injury and lowered anti-donor immune responses. In ABMR, complement is suggested to be activated by the classical pathway and to play a key role in the pathogenesis (Collard et al., 1997). Although the role of complement in HAR or acute vascular rejection (AVR) following xenotransplantation has been well documented (Platt et al., 1999), precise mechanisms of complement in the pathogenesis of ABMR following allotransplantation has not yet been elucidated.

Samsung Bioepis Exhibit No. 1002
Page 1292

The C5 component of complement is cleaved to form products with multiple proinflammatory effects and thus represents an attractive target for complement inhibition within the immune-mediated inflammatory response. As described above, C5a is a powerful anaphylatoxin and chemotactic factor. Cellular activation by C5a induces the release of multiple additional inflammatory mediators (Jose et al., 1983). The complement activation pathways (classical, alternative, or mannan-binding lectin pathway) ultimately lead to the formation of the cytolytic membrane attack complex C5b-9 (Kirschfunk, 2001), which can mediate both direct tissue injury by cell lysis, and proinflammatory cell activation at sublytic doses (Saadi et al., 1995; Papadimitriou et al., 1991). Therefore, blocking both C5a and C5b-9 generation may be required for the optimal inhibition of complement-mediated inflammatory response following transplantation. At the same time, inhibition of the complement cascade at C5 does not impair the generation of C3b, preserving C3b-mediated opsonization of pathogenic microorganisms as well as solubilization and clearance of immune complexes (Liszewski, 1993).

The beneficial effect of anti-C5 mAb has previously been reported in several experimental models including myocardial reperfusion (Vakeva et al., 1998), systemic lupus erythematosus (Wang et al., 1996) and rheumatoid arthritis (Wang et al., 1995); as well as in human clinical trials (Kirschfink, 2001) of autoimmune disease, cardiopulmonary bypass and acute myocardial infarction. In addition, complement inactivation by a functionally blocking anti-C5 monoclonal Ab (mAb) prevented HAR in xenotransplantation models (Kroshus et al., 1995; Wang et al., 1999).

Methods of delaying allotransplant rejection by administration of complement inhibitors have been tested. Published PCT patent application WO 92/10205 discloses the use of a combination of cyclosporin and a soluble complement receptor (sCR1) to inhibit rejection of a cardiac allotransplant in a presensitized rat model. Complement receptor 1 binds complements C3b and C4b. Soluble forms of complement receptor 1 occur naturally or can be generated via recombinant DNA procedures. These soluble complement receptors have inhibited in vitro the consequences of complement activation (U.S. Patent 6,057,131). In WO 92/10205, rats, which had been presensitized to the cardiac allograft they were receiving, were administered cyclosporin A intramuscularly at 10 mg/kg/day beginning two days prior to transplant and continued until the time of graft rejection. Additionally, soluble complement receptor 1 (sCR1) was administered as a single intravenous bolus at 15 mg/kg immediately prior to reperfusion of the graft. Control animals with no drug treatment had the

Samsung Bioepis Exhibit No. 1002
Page 1293

graft rejected at an average of 3.8 days. Those administered cyclosporin A alone rejected the grafts at an average of 57 days (this was quite variable with two rats rejecting quickly at 2 and 4 days and a third rat rejecting at 166 days). Rats administered sCR1 alone rejected the grafts at an average of 44 days. Those rats administered the combination of cyclosporin A and sCR1 rejected the grafts at an average of 147 days. The combination of chronic cyclosporin A and single bolus sCR1 was seen to result in a synergistic effect greatly prolonging the time until graft rejection. Earlier studies by Pruitt and Bollinger (1991) used a similar model of a presensitized rat allograft to show that administration of sCR1 alone to inactivate complement resulted in increased time before graft rejection.

Sims et al. (U.S. Patent 5,135,916) suggest using inhibitors of complement, e.g., CD59 or antibodies against C7 or C9 to block the formation of the C5b-9 complex, to treat the vascular endothelium of organs and tissues to be transplanted. This would prevent the C5b-9 initiated cell necrosis. The C5b-9 inactivators would be added to the perfusate or storage medium to protect the vascular lining cells from ongoing complement activation during in vitro storage. Additionally the organ or tissue would be protected from the cytolytic and thrombotic effects arising from complement activation initiated upon transplantation, thereby circumventing complement mediated acute rejection. Sims et al. (U.S. Patent 5,573,940 and U.S. Patent 6,100,443) also teach a method of expressing CD59 in the transplanted tissue or organ to protect the transplanted organ from rejection. This can be accomplished by transfecting the cells being transplanted.

Although the several drugs developed to date in combination with methods of prescreening donors and recipients to match the donor allograft to the recipient have over time increased the average length of time of survival of allografts, many allografts are nonetheless rejected during the life-time of the recipient. In general, the prior art advances have mainly been directed to overcoming acute graft rejection. Further, the role of activated terminal complement components in antibody-mediated allograft rejection has not been examined using inhibitors that specifically target the complement cascade at the C5 protein level. The methods described herein and as exemplified in the Examples advance the allotransplant art by inhibiting chronic rejection of allografts, in particular, allografts in a presensitized recipient. New methods are presented for further prolonging allograft survival by using a proper combination of immunosuppressive drugs in combination with a chronic administration of a complement inhibitor.

Samsung Bioepis Exhibit No. 1002
Page 1294

Methods and Uses

The methods disclosed herein are used to prolong allograft survival. The methods generally include administering an inhibitor of complement activity in combination with one or more immunosuppressants.

Suitable complement inhibitors are known to those of skill in the art. Antibodies can be made to individual components of activated complement, e.g., antibodies to C5a, C7, C9, etc. (see, e.g., U.S. Patent 6,534,058; published U.S. patent application US 2003/0129187; and U.S. Patent 5,660,825). Proteins are known which inhibit complement-mediated lysis, including CD59, CD55, CD46 and other inhibitors of C8 and C9 (see, e.g., U.S. Patent 6,100,443). U.S. Patent 6,355,245 teaches an antibody which binds to C5 and prevents it from being cleaved into C5a and C5b thereby preventing the formation not only of C5a but also the C5b-9 complex. Proteins known as complement receptors and which bind complement are also known (see, Published PCT Patent Application WO 92/10205 and U.S. Patent 6,057,131). Use of soluble forms of complement receptors, e.g., soluble CR1, can inhibit the consequences of complement activation such as neutrophil oxidative burst, complement mediated hemolysis, and C3a and C5a production. Those of skill in the art recognize the above as some, but not all, of the known methods of inhibiting complement and its activation.

Suitable immunosuppressants include, but are not limited to, ATG or ALG, OKT3, daclizumab, basiliximab, corticosteroids, 15-deoxyspergualin, cyclosporins, tacrolimus, azathioprine, methotrexate, mycophenolate mofetil, 6-mercaptopurine, bredinin, brequinar, leflunamide, cyclophosphamide, sirolimus, anti-CD4 monoclonal antibodies, CTLA4-Ig, anti-CD154 monoclonal antibodies, anti-LFA1 monoclonal antibodies, anti-LFA-3 monoclonal antibodies, anti-CD2 monoclonal antibodies, and anti-CD45.

An allograft can include a transplanted organ, part of an organ, tissue or cell. These include, but are not limited to, heart, kidney, lung, pancreas, liver, vascular tissue, eye, cornea, lens, skin, bone marrow, muscle, connective tissue, gastrointestinal tissue, nervous tissue, bone, stem cells, islets, cartilage, hepatocytes, and hematopoietic cells.

At least part of the reason for the failure of allografts is that one response by the recipient of an allograft is the activation of complement. This results in the formation of C5a and C5b-9 which are potent proinflammatory molecules which aid in causing graft failure. Without wishing to be bound by any proposed theory, Applicants theorized that inhibiting the

Samsung Bioepis Exhibit No. 1002
Page 1295

formation of C5a and C5b-9 or inhibiting C5a and C5b-9 which was present would aid in preventing graft failure. Furthermore, it was theorized that so long as the allograft is present, the recipient will continue to attempt to mount an immune response against the graft, and this response will include attempts to produce C5a and C5b-9. If not prevented, this complement response will lead to acute vascular rejection in the short term and could contribute to chronic graft rejection in the long term. Prior art methods of using inhibitors of complement activity were limited to administering these inhibitors only at the time of transplant. This was helpful in preventing acute rejection, but as the results disclosed herein illustrate, improved results are obtained by administration of such inhibitors for a longer term. This long-term administration aids in preventing a chronic rejection of the allograft as opposed to only aiding in preventing an acute rejection. The result is a longer term survival of the allograft as compared to either not administering an inhibitor of complement activity or administering such an inhibitor only at the time of transplant of the allograft. Although very commonly it is desirable that the allograft will survive for the remaining lifetime of the recipient, there are times when the allograft is needed only for a shorter length of time, e.g., a bridge organ to bridge the time until the recipient's own organ can recover on its own, at which time the allograft will no longer be needed. The length of time such a graft will be needed will vary, but will usually be longer than the time at which acute rejection would occur and may be long enough for chronic rejection to occur. This period of desired survival for a bridge graft may be several months, e.g., six months.

To prove that long-term inhibition of complement activity will prolong allograft survival, experiments were performed in which complement activation was inhibited in a chronic fashion and not merely at the time of transplant. Chronic treatment means treatment during an extended period up to the lifetime of the allograft. This can be daily treatment but is not limited to daily treatment. Chronic treatment will maintain an effective amount of the drug in the allograft recipient. For example, a preferred method is to include the anti-C5 monoclonal antibody eculizumab in the treatment. In studies of persons suffering from paroxysmal nocturnal hemoglobinuria (PNH), eculizumab has been administered at a dose of 900 mg/patient once every 12-14 days. This dosing has been found to completely and consistently block terminal complement activity and has greatly inhibited the symptoms of PNH (Hillmen et al., 2004). The administered dose is able to block the effects of complement for approximately two weeks before the eculizumab is inactivated or removed from the body. Therefore, a chronic treatment of eculizumab may be, e.g., the administration

Samsung Bioepis Exhibit No. 1002
Page 1296

of 900 mg to the allograft recipient once every two weeks for the remaining life-time of the patient. Similarly, other drugs can be delivered chronically as needed, whether this is on a daily basis or another schedule is required to maintain an effective amount of the drug in the allograft recipient. Because it is well known that graft rejection can be caused by more than just complement activation, e.g., by T cell activity, the experiments included immunosuppressants such as cyclosporin to further aid in preventing graft rejection.

A preferred method of inhibiting complement activity is to use a monoclonal antibody which binds to complement C5 and prevents C5 from being cleaved. This prevents the formation of both C5a and C5b-9 while at the same time allowing the formation of C3a and C3b which are beneficial to the recipient. Such antibodies that are specific to human complement are known (U.S. Patent 6,355,245). These antibodies disclosed in U.S. Patent 6,355,245 include both a whole or full-length antibody (now named eculizumab) and a single-chain antibody (now named pexelizumab). A similar antibody against mouse C5 is called BB5.1 (Frei et al., 1987). BB5.1 was utilized in the experiments set forth below. Antibodies to inhibit complement activity need not be monoclonal antibodies. They can be, e.g., polyclonal antibodies. They may additionally be antibody fragments. An antibody fragment includes, but is not limited to, an Fab, F(ab'), $F(ab')_2$, a single-chain antibody, a domain antibody and an Fv. Furthermore, it is well known by those of skill in the art that antibodies can be humanized (Jones et al., 1986), chimerized, or deimmunized. An antibody may also comprise a mutated Fc portion, such that the mutant Fc does not activate complement. The antibodies to be used in the present disclosure may be any of these. It is preferable to use humanized antibodies when the recipient of the allograft is a human.

Administration and Formulations

Administration of the inhibitor of complement activity is performed according to methods known to those of skill in the art. These inhibitors are administered preferably before the time of allograft transplantation or at the time of transplantation with administration continuing in a chronic fashion. These inhibitors can additionally be administered during a rejection episode in the event such an episode does occur.

The present disclosure also provides uses of a drug that inhibits complement activity and an immunosuppressive agent in the manufacture of a medicament or medicament package. Such medicament or medicament package is useful in prolonging allograft survival in a recipient, in particular, chronic survival of the allograft. In preferred embodiments, the

Samsung Bioepis Exhibit No. 1002
Page 1297

medicament or medicament package is formulated and prepared such that it is suitable for chronic administration to the recipient of the allograft, for example, stable formulations are employed. In certain embodiments, the medicament or medicament package is formulated and prepared such that it is suitable for concurrent administration of the drug that inhibits complement activity and the immunosuppressive drug to the recipient of the allograft. In certain embodiments, the medicament or medicament package is formulated and prepared such that it is suitable for sequential (in either order) administration of the drug that inhibits complement activity and the immunosuppressive drug to the recipient of the allograft.

A pharmaceutical package of the present disclosure may comprise a drug that inhibits complement activity and at least one immunosuppressive agent. The pharmaceutical package may further comprise a label for chronic administration. The pharmaceutical package may also comprise a label for self-administration by a patient, for example, a recipient of a transplant graft, or instructions for a caretaker of a recipient of a transplant graft. In certain embodiments, the drug and the agent in the pharmaceutical package are in a formulation or separate formulations that are suitable for chronic administration and/or self-administration.

The present disclosure also provides lyophilized formulations and formulations suitable for injection. Certain embodiments provide a lyophilized antibody formulation comprising an antibody that inhibits complement activity and a lyoprotectant. In preferred embodiments, the antibody formulation is suitable for chronic administration, for example, the antibody formulation stable. Alternative embodiments provide an injection system comprising a syringe; the syringe comprises a cartridge containing an antibody that inhibits complement activity and is in a formulation suitable for injection.

An antibody employed in various embodiments of the present disclosure preferably inhibits the formation of terminal complement or C5a. In certain embodiments, antibody inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment. The whole antibody or antibody fragment may be a human, humanized, chimerized or deimmunized antibody or antibody fragment. In certain embodiments, the whole antibody or antibody fragment may inhibit cleavage of complement C5. In certain embodiments, the antibody fragment is a Fab, an F(ab')2, an Fv, a domain antibody, or a single-chain antibody. In preferred embodiments, the antibody fragment is pexelizumab. In alternative preferred embodiments, the whole antibody is eculizumab.

Samsung Bioepis Exhibit No. 1002
Page 1298

In certain embodiments, a drug, such as an antibody, that inhibits complement activity is present in unit dosage form, which can be particularly suitable for self-administration. Similarly, an immunosuppressive agent of the present disclosure may also be present in unit dosage form. A formulated product of the present disclosure can be included within a container, typically, for example, a vial, cartridge, prefilled syringe or disposable pen. A doser such as the doser device described in U.S. Patent 6,302,855 may also be used, for example, with an injection system of the present disclosure.

A "stable" formulation is one in which the drug (e.g., an antibody) or agent therein essentially retains its physical and chemical stability and integrity upon storage. Various analytical techniques for measuring protein stability are available in the art and are reviewed in Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. Adv. Drug Delivery Rev. 10: 29-90 (1993). Stability can be measured at a selected temperature for a selected time period. For example, the extent of aggregation following lyophilization and storage can be used as an indicator of protein stability. For example, a "stable" formulation may be one wherein less than about 10% and preferably less than about 5% of the protein is present as an aggregate in the formulation. In other embodiments, any increase in aggregate formation following lyophilization and storage of the lyophilized formulation can be determined. For example, a "stable" lyophilized formulation may be one wherein the increase in aggregate in the lyophilized formulation is less than about 5% and preferably less than about 3%, when the lyophilized formulation is stored at 2-8 °C for at least one year. In other embodiments, stability of the protein formulation may be measured using a biological activity assay.

A "reconstituted" formulation is one which has been prepared by dissolving a lyophilized protein formulation in a diluent such that the protein is dispersed in the reconstituted formulation. The reconstituted formulation in suitable for administration (e.g. parenteral administration) to a patient to be treated with the protein of interest and, in certain embodiments of the invention, may be one which is suitable for subcutaneous administration.

An isotonic reconstituted formulation is preferable in certain embodiments. By "isotonic" is meant that the formulation of interest has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 250 to 350 mOsm. Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example.

Samsung Bioepis Exhibit No. 1002
Page 1299

A "lyoprotectant" is a molecule which, when combined with a drug (e.g., antibody) of interest, significantly prevents or reduces chemical and/or physical instability of the drug (e.g., antibody) upon lyophilization and subsequent storage. Exemplary lyoprotectants include sugars such as sucrose or trehalose; an amino acid such as monosodium glutamate or histidine; a methylamine such as betaine; a lyotropic salt such as magnesium sulfate; a polyol such as trihydric or higher sugar alcohols, e.g. glycerin, erythritol, glycerol, arabitol, xylitol, sorbitol, and mannitol; propylene glycol; polyethylene glycol; Pluronics; and combinations thereof. The preferred lyoprotectant is a non-reducing sugar, such as trehalose or sucrose.

The lyoprotectant is added to the pre-lyophilized formulation in a "lyoprotecting amount" which means that, following lyophilization of the drug (e.g., antibody) in the presence of the lyoprotecting amount of the lyoprotectant, the drug (e.g., antibody) essentially retains its physical and chemical stability and integrity upon lyophilization and storage.

The "diluent" of interest herein is one which is pharmaceutically acceptable (safe and non-toxic for administration to a human) and is useful for the preparation of a reconstituted formulation. Exemplary diluents include sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (e.g. phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

A "preservative" is a compound which can be added to the diluent to essentially reduce bacterial action in the reconstituted formulation, thus facilitating the production of a multi-use reconstituted formulation, for example. Examples of potential preservatives include octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride (a mixture of alkylbenzyldimethylammonium chlorides in which the alkyl groups are long-chain compounds), and benzethonium chloride. Other types of preservatives include aromatic alcohols such as phenol, butyl and benzyl alcohol, alkyl parabens such as methyl or propyl parahen, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol.

A "bulking agent" is a compound which adds mass to the lyophilized mixture and contributes to the physical structure of the lyophilized cake (e.g. facilitates the production of an essentially uniform lyophilized cake which maintains an open pore structure). Exemplary bulking agents include mannitol, glycine, polyethylene glycol and xorbitol.

Accordingly, a stable lyophilized antibody formulation can be prepared using a lyoprotectant (preferably a sugar such as sucrose or trehalose), which lyophilized formulation

Samsung Bioepis Exhibit No. 1002
Page 1300

can be reconstituted to generate a stable reconstituted formulation having an antibody concentration which is significantly higher (e.g. from about 2-40 times higher, preferably 3-10 times higher and most preferably 3-6 times higher) than the antibody concentration in the pre-lyophilized formulation. Such high protein concentrations in the reconstituted formulation are considered to be particularly useful where the formulation is intended for subcutaneous administration. Despite the very high protein concentration in the reconstituted formulation, the reconstituted formulation can be stable (i.e. fails to display significant or unacceptable levels of chemical or physical instability of the protein) at 2-8 °C. for at least about 30 days. See U.S. Patent 6,821,515. In certain embodiments, the reconstituted formulation is isotonic.

When reconstituted with a diluent comprising a preservative (such as bacteriostatic water for injection, BWFI), the reconstituted formulation may be used as a multi-use formulation. Such a formulation is useful, for example, where a subject patient requires frequent administrations of the drug or antibody and/or agent to treat a chronic medical condition. The advantage of a multi-use formulation is that it facilitates ease of use for the patient, reduces waste by allowing complete use of vial contents, and results in a significant cost savings for the manufacturer since several doses are packaged in a single vial (lower filling and shipping costs).

The present disclosure also provides a method for preparing a formulation comprising the steps of: (a) lyophilizing a mixture of an antibody and a lyoprotectant; and (b) reconstituting the lyophilized mixture of step (a) in a diluent such that the reconstituted formulation is isotonic and stable.

An article of manufacture is also provided herein which comprises: (a) a container which holds a lyophilized mixture of an antibody and a lyoprotectant; and (b) instructions for reconstituting the lyophilized mixture with a diluent to a desirable antibody concentration in the reconstituted formulation. The article of manufacture may further comprise a second container which holds a diluent (e.g. bacteriostatic water for injection (BWFI) comprising an aromatic alcohol).

An injection system of the present disclosure may employ a medication delivery pen as described in U.S. Patent 5,308,341. Medication delivery pens have been developed to facilitate the self-administration of medication. A medication of the present disclosure can be a drug that inhibits complement activity, for example an antibody specific to complement C5,

Samsung Bioepis Exhibit No. 1002
Page 1301

and/or an immunosuppressive agent. One medication delivery pen includes a vial holder into which a vial of insulin or other medication may be received. The vial holder is an elongate generally tubular structure with proximal and distal ends. The distal end of the vial holder includes mounting means for engaging a double-ended needle cannula. The proximal end also includes mounting means for engaging a pen body which includes a driver and dose setting apparatus. A disposable medication containing vial for use with the prior art vial holder includes a distal end having a pierceable elastomeric septum that can be pierced by one end of a double-ended needle cannula. The proximal end of this vial includes a stopper slidably disposed in fluid tight engagement with the cylindrical wall of the vial. This medication delivery pen is used by inserting the vial of medication into the vial holder. A pen body then is connected to the proximal end of the vial holder. The pen body includes a dose setting apparatus for designating a dose of medication to be delivery by the pen and a driving apparatus for urging the stopper of the vial distally for a distance corresponding to the selected dose.

The user of the pen mounts a double-ended needle cannula to the distal end of the vial holder such that the proximal point of the needle cannula pierces the septum on the vial. The patient then selects a dose and operates the pen to urge the stopper distally to deliver the selected dose. The dose selecting apparatus returns to zero upon injection of the selected dose. The patient then removes and discards the needle cannula, and keeps the prior art medication delivery pen in a convenient location for the next required medication administration. The medication in the vial will become exhausted after several such administrations of medication. The patient then separates the vial holder from the pen body. The empty vial may then be removed and discarded. A new vial can be inserted into the vial holder, and the vial holder and pen body can be reassembled and used as explained above.

Accordingly, a medication delivery pen generally has a drive mechanism for accurate dosing and ease of use. A dosage mechanism such as a rotatable knob allows the user to accurately adjust the amount of medication that will be injected by the pen from a prepackaged vial of medication. To inject the dose of medication, the user inserts the needle under the skin and depresses the knob once as far as it will depress. The pen may be an entirely mechanical device or it may be combined with electronic circuitry to accurately set and/or indicate the dosage of medication that is injected into the user. See U.S. Patent 6,192,891.

Samsung Bioepis Exhibit No. 1002
Page 1302

The present disclosure also presents controlled-release or extended-release formulations suitable for chronic and/or self-administration of a medication.

The various formulations can be administered to a patient in need of treatment (e.g., a recipient of an allograft) with the medication (e.g., an antibody of the present disclosure and at least one immunosuppressive agent) by intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

In certain embodiments, a formulation is administered to the patient by subcutaneous (i.e. beneath the skin) administration. For such purposes, the formulation may be injected using a syringe. However, other devices for administration of the formulation are available such as injection devices (e.g. the Inject-ease® and Genject® devices); injector pens (such as the GenPen®); needleless devices (e.g. MediJector® and BioJector®); and subcutaneous patch delivery systems.

The present methods and uses are described with reference to the following Examples, which are offered by way of illustration and are not intended to limit the disclosure in any manner. Standard techniques well known in the art or the techniques specifically described below are utilized. The following abbreviations are used herein: ABMR, antibody-mediated rejection; ACHR, accelerated humoral rejection; ACR, acute cellular rejection; AVR, acute vascular rejection; CsA, cyclosporin; CyP, cyclophosphamide; HAR, hyperacute rejection; MCP-1, monocyte chemotactic protein 1; MST, mean survival time; POD, postoperative day.

Example 1

Methods

Animals and Immunosuppressive Drugs Male adult C3H (H-2^k) mice and BALB/c (H-2^d) mice (Jackson Labs, Bar Harbor, Maine) weighing 25-30 g were chosen as donors and recipients, respectively. In the groups receiving immunosuppression, the recipients were injected with CsA (15 mg/kg/day, s.c., daily from day 0 to endpoint rejection or until day 100), or with CyP (40 mg/kg/day, i.v., on day 0 and 1), or with anti-C5 mAb (clone BB5.1, Alexion Pharmaceuticals Inc., 40 mg/kg/day, i.p., day 0–2, followed by twice a week, day 0-60). Animals were housed under conventional conditions at the Animal Care Facility,

Samsung Bioepis Exhibit No. 1002
Page 1303

University of Western Ontario, and were cared for in accordance with the guidelines established by the Canadian Council on Animal Care. Olfert et al., 1993.

Skin Presensitization Full-thickness skin grafts taken from C3H donors were cut into square pieces of 1×1 cm^2 and transplanted onto the back of the BALB/c recipients' thorax one week prior to heart transplantation from the same donors. Rejection was defined as complete necrosis of the skin grafts.

Abdominal and Cervical Cardiac Transplantation Seven days after skin presensitization, C3H mouse hearts were transplanted into the abdomen of presensitized BALB/c recipients by anastomosing the donor aorta and recipient aorta, and the donor pulmonary artery and recipient inferior vena cava. In the groups with re-transplantation, second heart grafts harvested from either naive C3H mice or long-term surviving presensitized BALB/c recipients were transplanted into the cervical area of the recipients carrying a long-term surviving first abdominal heart graft by anastomosing the donor aorta and recipient carotid artery, and the donor pulmonary artery and recipient external jugular vein (end-to-side). The heart grafts were monitored daily until rejection unless otherwise indicated and rejection was defined as complete cessation of pulsation.

Experimental Groups Presensitized recipients were randomly assigned to eight groups, each consisting of eight animals: Group 1, mice with no treatment; Group 2, mice treated with CsA; Group 3, mice treated with CyP; Group 4, mice treated with CsA plus CyP; Group 5, mice treated with anti-C5 mAb; Group 6, mice treated with anti-C5 mAb plus CsA; Group 7, mice treated with anti-C5 mAb plus CyP; Group 8, mice treated with anti-C5 mAb in combination of CsA and CyP. When cardiac impulses were no longer palpable or at POD100, the grafts were removed for routine histology, immunohistochemistry and western blot analysis, serum samples were collected for flow cytometric analysis and complement hemolytic assay. Five additional animals were placed and sacrificed in groups 6 and 8 on POD3 (MST for groups 1-5, 7) to allow for comparisons at a uniform time point. Serum samples were also collected on POD 11, 21, 28 and 60 in Group 8 for detecting the sequential changes of anti-donor antibody levels and complement activity. In addition, when triple therapy treated presensitized recipients carried a first heart graft for 100 days, they were re-transplanted with a second heart. A naive C3H heart or a 100-day surviving C3H heart from another presensitized BALB/c recipient was used as the second heart. Eight animals were included in each re-transplant group.

Samsung Bioepis Exhibit No. 1002
Page 1304

Graft Histology Tissue samples were fixed in 10% buffered formaldehyde. Specimens were then embedded in paraffin, and sectioned for H&E staining. The microscopic sections were examined in a blinded fashion for severity of rejection by a pathologist. Criteria for graft rejection included the presence of vasculitis, thrombosis, hemorrhage and lymphocyte infiltration. These changes were scored as: 0, no change; 1, minimum change; 2, mild change; 3, moderate change; or 4, marked change.

Immunohistochemistry Four micrometer sections were cut from tissue samples embedded in Tissue-Tek O.C.T gel (Optimum Cutting Temperature, Skura Finetek, Torrance, CA) mounted on gelatin-coated glass microscope slides and stained by a standard indirect avidin-biotin immunoperoxidase staining method using an Elite Vectastain ABC kit (Vector Laboratories Inc., Burlingame, CA). Specimens were stained for $CD4^+$ and $CD8^+$ cells with biotin-conjugated rat anti-mouse CD4 mAb (clone YTS 191.1.2, Cedarlane Laboratories Ltd., Hornby, Ontario, Canada) and biotin-conjugated rat anti-mouse CD8 mAb (clone 53-6.7, Pharmingen, Franklin Lakes, NJ), respectively. Intragraft monocyte/macrophage infiltration was detected by staining with biotin-conjugated rat anti-mouse Mac-1 mAb (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada). Mouse IgG and IgM deposition in grafts was detected using biotin-conjugated goat anti-mouse-IgG and goat anti-mouse-IgM (Cedarlane). For identification of complement deposition, sections were serially incubated with goat anti-C3 or anti-C5 polyclonal Abs (Quidel, San Diego, CA), biotinylated rabbit anti-goat IgG (Vector Laboratories), and HRP-conjugated-streptavidin (Zymed Laboratories, South San Francisco, CA). Slides were washed with phosphate-buffered saline between steps, and examined under light microscopy. Negative controls were performed by omitting the primary antibodies. The immunostaining was scored in five high-power fields of each section, and five independent experiments were performed. The sections of immunoperoxidase staining were graded from 0 to 4+ according to the staining intensity: 0, negative; 1+, equivocal; 2+, weak staining; 3+, moderate staining; and 4+, very intensive staining.

Flow Cytometry The circulating anti-donor specific IgG and IgM antibodies were evaluated in the recipient serum by FACScan flow cytometry (Becton Dickinson, Mountain View, CA). Glotz et al., (1993); Tyan et al. (1994). Briefly, C3H mouse splenocytes were isolated and incubated at 37 °C for 30 minutes with serum from naive control and experimental groups. To stain for total IgG, IgG1, IgG2a, IgG2b and IgM, the cells were washed and incubated with FITC-conjugated goat antibody specific for the Fc portion of mouse IgG or with

Samsung Bioepis Exhibit No. 1002
Page 1305

phycoerythrin-conjugated goat antibody specific for the μ-chain of mouse IgM (Jackson ImmunoResearch Laboratories, West Grove, PA), or with FITC-conjugated goat anti-mouse IgG1 (CALTAG Laboratories, Burlingame, CA), or with FITC-conjugated goat anti-mouse IgG2a (CALTAG), or with FITC-conjugated goat anti-mouse IgG2b (CALTAG). After 1 hour of staining at 4 °C, the cells were washed with PBS, resuspended at 5×10^6/mL, and analyzed by flow cytometry for mean channel fluorescence intensity, which represents the antibody-binding reactivity.

Complement Hemolytic Assay The purified anti-C5 mAb was serially diluted twofold (175-0.1 μg/ml) in GVB^{2+} buffer (gelatin Veronal-buffered saline: 0.1% gelatin, 141 mM NaCl, 0.5 mM $MgCl_2$, 0.15 mM $CaCl_2$, and 1.8 mM sodium barbital) and added in triplicate (50 μl/well) to a 96-well plate. BALB/c mouse serum was diluted to 40% v/v with GVB^{2+} buffer and added (50 μl/ml) to the rows of the same 96-well plate such that the final concentration of BALB/c mouse serum in each well was 20%. The plate was then incubated at room temperature for approximately 30 min while chicken erythrocytes were prepared. Chicken erythrocytes were washed 5×1 ml with GVB^{2+} buffer and resuspended to a final concentration of 5×10^7/ml in GVB^{2+}. Four milliliters of the chicken erythrocytes were sensitized by adding anti-chicken RBC polyclonal antibody (Intercell Technologies, Hopewell, NJ, 0.1% v/v) and the cells were incubated at 4 °C for 15 min with frequent vortexing. The cells were then washed 2×1 ml with GVB^{2+} and resuspended to a final volume of 2.4 ml in GVB^{2+}. The chicken erythrocytes (30 μl/well, 2.5×10^6 cells) were added to the plate containing serum and anti-C5 mAb as described above, mixed well, and incubated at 37 °C for 30 min. The plate was then centrifuged at 1000×g for 2 min, and 85 μl of the supernatant was transferred to a new 96-well microtiter plate. The plate was read at OD 415 nm using a microplate reader and the percentage of hemolysis was determined using this formula:

$$\%\ \text{hemolysis} = 100 \times \frac{(\text{OD sample}) - (\text{OD } GVB^{2+} \text{ control})}{(\text{OD 100\% lysed control}) - (\text{OD } GVB^{2+} \text{ control})}$$

with 100% lysed control obtained by the addition of 100 μl GVB^{2+} containing 0.1% NP-40 to the 30 μg/ml of chicken erythrocytes as prepared above.

Samsung Bioepis Exhibit No. 1002
Page 1306

Western Blot Analysis Sonication of frozen heart samples was performed in RIPA lysis buffer (Santa Cruz Biotechnology, Inc.) at 4 °C for 1 minute at 10-second intervals, followed by microcentrifugation at 13,000 rpm for 10 minutes at 4 °C. Clarified supernatants were immediately quantitated in triplicate for protein content using Detergent-compatible protein assay kit (BIO-RAD). Heart lysates (10μg protein/well) were separated on NuPAGE, 4-12% gradient Bis-Tris gels and MES buffer system (Invitrogen) and transferred to polyvinylidene difluoride (PVDF) membrane (0.45 μm pore size; Invitrogen) using a semi-dry transfer apparatus (BIO-RAD). Membranes were cut appropriately at the correct molecular weights to allow the development of the blots with two different primary antibodies per blot such that each blot was exposed to a test antibody and an internal control antibody to insure equal sample loading. The test primary antibodies including anti-Bcl-2 (N-19) rabbit polyclonal sera (Santa Cruz Biotechnology, Inc.) and anti-Bcl-XS/L (M-125) rabbit polyclonal sera (Santa Cruz Biotechnology, Inc.) were used to detect intragraft expression of Bcl-2 and Bcl-xl proteins. Anti-calsequestrin rabbit polyclonal sera (Calbiochem) were used as internal control primary antibody (Kobayashi et al., 1999). Detection of primary antibody binding was performed as previously described (Arp et al., 1996) by exposing washed incubated blots to a polyclonal goat anti-rabbit IgG fraction conjugated to horseradish peroxidase (HRP; Roche Laboratories) and then appropriately developed by exposure to enhance chemiluminescence for HRP-conjugated antibodies (Roche Laboratories).

Statistical Analysis The data were reported as the mean ± SD. Allograft survival among experimental groups was compared using the rank-log test. Histological and immunohistological findings were analyzed using the Mann-Whitney U test. Flow cytometric data and western blot data were analyzed using one-way ANOVA. Differences with p values less than 0.05 were considered significant.

Example 2

Presensitization with C3H donor skin graft induces antibody-mediated ACHR in heart allografts of BALB/c recipients.

To develop a suitable small animal model that mimics presensitized patients in the clinic and to study ABMR, a novel, fully MHC-mismatched mouse ABMR model has been developed through presensitization of mouse recipients. In this model, BALB/c recipients were presensitized with C3H donor skin grafts one week prior to heart transplantation from the same donor. Seven days after donor skin presensitization, serum level of anti-donor IgG,

Samsung Bioepis Exhibit No. 1002
Page 1307

but not IgM antibody was markedly elevated and reached to a peak level in the presensitized BALB/c recipients (Figure 1A). Heart transplantation from same donor was then performed in these highly sensitized recipients. Without immunosuppression, C3H heart grafts were rapidly rejected in 3.1 ± 0.4 days by ACHR, characterized by severe thrombosis, hemorrhage and infarction (Figure 1B-a). In contrast, same heart grafts in unsensitized BALB/c recipients (with mean survival time, MST of 8.2 ± 0.8 days) show the normal histology on post-operative day (POD) 3 (Figure 1B-b). When compared to unsensitized BALB/c recipients at the same day, heart grafts in presensitized animals revealed massive IgG antibody and complement (C3 and C5) deposition, but minimal $CD4^+$ and $CD8^+$ cell infiltration (Table 1). Furthermore, circulating anti-donor IgG levels in presensitized recipients were significantly higher than those of unsensitized same recipients receiving a heart graft on POD3 ($P< 0.01$, Figure 1C). However, anti-donor IgM remained at very low levels both in circulation (Figure 1C) and in heart grafts (Table 1) and it showed no significant difference between unsensitized and presensitized recipients. In addition, normal levels of complement hemolytic activity were shown in both presensitized and unsensitized heart recipients without treatment (Figure 1D). These data indicate that this is an ideal transplant model to study ABMR in presensitized recipients in which complement plays an important role in the pathogenesis.

Table 1.

Comparison of immunohistological changes of C3H heart allografts in unsensitized and presensitized BALB/c recipients on POD3

Groups	Unsensitized	Presensitized
IgG	1+	4+
IgM	1+	1+
C3	2+	3+
C5	2+	3+
CD4	0	1+
CD8	0	1+

Samsung Bioepis Exhibit No. 1002
Page 1308

Grades for immunoperoxidase staining: 0, negative; 1+, equivocal; 2+, weak; 3+, moderate; 4+, intense.

Anti-C5 mAb in combination with CsA and CyP prevents ABMR and achieves indefinite heart allograft survival in presensitized mouse recipients.

Complement has been shown to play an important role in ABMR. However, the inhibitory effect of functionally blocking terminal complement cascade at the C5 level in highly sensitized recipients is unknown. In the study presented herein, the presensitized model was used to study the efficacy of anti-C5 mAb either alone or combined with CsA and /or CyP in prevention of ABMR. As presented in Figure 2A, treatment with either CsA or CyP or the two drugs in combination did not prevent ABMR and grafts were rejected in 3.0 ± 0.0 days, 3.3 ± 0.5 days and 3.5 ± 0.6 days, respectively with typical pathological features of ACHR including intravascular thrombosis and interstitial hemorrhage (Figure 2B-b, c, d), which were indistinguishable from heart grafts in untreated presensitized BALB/c recipients (Figure 2B-a). Anti-C5 monotherapy or combined with CyP was not able to improve graft survival and heart grafts were rejected by ACHR (Figure 2B-e, f) in 3.5 ± 0.6 days and 3.2 ± 0.4 days, respectively (Figure 2A). Although the combination therapy of anti-C5 mAb and CsA, the protocol capable of inducing long-term heart allograft survival in unsensitized animals, marginally prolonged graft survival in this presensitized model, heart grafts were also rejected by severe humoral rejection with vasculitis, thrombosis, hemorrhage and minimal cell infiltration (Figure 2B-g) in 11.9 ± 1.8 days (Figure 2A). In contrast, triple therapy of anti-C5 mAb in combination of CsA and CyP achieved indefinite heart graft survival over 100 days (Figure 2A) in presensitized animals ($P<0.01$ vs. the animals without treatment or treated with either monotherapy or two drugs in combination) with no evidence of rejection (Figure 2B-h). In this presensitized mouse model, as shown in Table 2, only minor intragraft $CD4^+$ and $CD8^+$ cell infiltration was observed in the recipients that rejected their heart grafts within 3 days. However, the number of these T cells was slightly increased if heart grafts survived longer in anti-C5 mAb plus CsA-treated recipients at the time of rejection (POD11) and in triple therapy-treated recipients at early stages of graft survival (e.g. POD11). Furthermore, with continuous treatment of CsA in the triple therapy group, $CD4^+$ and $CD8^+$ cell infiltration was inhibited in long-term surviving heart grafts on POD60 and 100. In addition, moderate intragraft Mac-1^+ cell infiltration, including monocytes and macrophages, was found in untreated and CsA-, CyP- or CsA plus CyP-treated animals,

Samsung Bioepis Exhibit No. 1002
Page 1309

while the infiltration of these cells was significantly reduced in anti-C5 mAb treated animals (Table 2). These results indicate that functionally blocking anti-C5 mAb enables the use and efficacy of conventional immunosuppressive agents, thereby preventing ABMR and achieving indefinite heart graft survival in presensitized recipients.

Table 2.

Grades for immunoperoxidase staining of heart allografts

in presensitized mouse recipients at necropsy

Groups	Date for sample collection (POD)	C3	C5	**CD4**	CD8	Mac-1	IgG	IgM
Untreated	3	3+	3+	1+	1+	3+	4+	1+
CsA	3	3+	3+	1+	1+	3+	4+	1+
CyP	3	3+	3+	1+	1+	3+	3+	1+
CsA+CyP	3	3+	3+	1+	1+	3+	3+	1+
Anti-C5mAb	3	3+	0	1+	1+	2+	4+	1+
Anti-C5mAb+CsA	11	3+	0	2+	2+	2+	4+	1+
Anti-C5mAb+CyP	3	3+	0	1+	1+	2+	3+	1+
Anti-C5mAb+CsA+CyP	3	3+	0	1+	1+	2+	3+	1+
Anti-C5mAb+CsA+CyP	11	3+	0	2+	2+	1+	3+	1+
Anti-C5mAb+CsA+CyP	60	3+	0	1+	1+	0	2+	1+
Anti-C5mAb+CsA+CyP	100	3+	2+	0	0	0	2+	1+

Grades: 0, negative; 1+, equivocal; 2+, weak; 3+, moderate; 4+, intense.

Anti-C5 mAb completely inhibits total complement hemolytic activity and local C5 deposition in presensitized recipients receiving a heart allograft.

Anti-C5 mAb was previously shown to block the cleavage of complement protein C5 into the proinflammatory molecules C5a and C5b-9 (Kroshus et al., 1995), and to completely and consistently block terminal complement activity in mice (Wang et al., 1999). In the current study, terminal complement activity was measured by assessing the ability of

Samsung Bioepis Exhibit No. 1002
Page 1310

recipient mouse sera to lyse antibody presensitized chicken erythrocytes and was compared at the same time-point (POD3). Treatment of mice with either CsA or CyP or the two drugs in combination had no effect on terminal complement activity, while treatment with anti-C5 mAb either alone or combined with CsA or/and CyP completely inhibited this activity (Figure 3; $P<0.01$, vs. naive and untreated animals, as well as CsA-, CyP-, or CsA plus CyP-treated animals). In addition, sera obtained from anti-C5 mAb treated animals at several earlier time points showed similarly diminished hemolytic activity, suggesting that serum terminal complement was inhibited throughout the treatment period. Furthermore, local C5 deposition in heart grafts was completely prevented in the anti-C5 mAb treated presensitized recipients, but not in untreated, or CsA-, CyP- and CsA plus CyP-treated presensitized animals (Table 2). As predicted, treatment with anti-C5 mAb did not prevent C3 deposition in the grafts (Table 2). These results suggest anti-C5 therapy completely blocks total complement activity after cardiac allografting in highly sensitized recipients.

Long-term surviving heart grafts in presensitized animals are resistant to humoral injury in the presence of low level of anti-donor antibodies and complement- a situation of accommodation.

To further investigate the role of anti-C5 mAb in humoral rejection, anti-donor alloantibody levels were measured in recipient sera by flow cytometry and intragraft antibody deposition by using immunostaining techniques in different groups. Figure 4A shows that on POD3 untreated presensitized BALB/c recipients had high levels of circulating anti-donor IgG antibodies. When presensitized recipients receiving either monotherapy or two drugs in combination, CsA and/or CyP partially down-regulated circulating anti-donor IgG levels, while treatment with anti-C5 mAb either alone or combined with CsA or CyP did not further affect anti-donor antibody levels at the same day. In contrast, with triple therapy of anti-C5 mAb, CsA and CyP, a high level of circulating anti-donor IgG was gradually downregulated and reached a low level on POD60, thereafter remaining at this level until day 100 (Figure 4B). Similar to levels of circulating antibodies in the different treatment groups, Table 2 shows that strong deposition of anti-mouse IgG was present in the rapidly rejected heart grafts of presensitized animals with no treatment or treated with monotherapy or two drugs in combination therapy. Interestingly, with triple therapy, IgG antibody deposition was gradually attenuated to a mild level in the long-term surviving heart grafts on POD 100 (Figure 4C-a, Table 2). In this model, IgM remained at very low levels in either circulation (Figure 4A, B) or transplanted heart grafts (Figure 4C-b, Table 2) in presensitized recipients

Samsung Bioepis Exhibit No. 1002
Page 1311

with or without treatment. In addition, treatment with anti-C5 mAb eliminated complement activity to an undetectable level until day 60, followed by a progressive recovery to predepletion levels on POD 100 after discontinuation of anti-C5 therapy in presensitized mouse recipients receiving triple therapy (Figure 4D). Furthermore, intragraft C5 deposition was also detected in 100-day surviving presensitized animals (Table 2). These data demonstrate that ongoing transplant accommodation occurs in triple therapy treated presensitized recipients despite the presence of anti-graft antibodies and complement activation.

Anti-C5 mAb in combination with CsA and CyP reduces the IgG1/IgG2a ratio and leads to a shift in IgG subclass to IgG2b in recipients with accommodated grafts.

To determine whether anti-C5 mAb-based triple therapy would induce a shift in IgG subclass, which may be associated with accommodation, serum levels of anti-donor IgG subclasses of IgG1, IgG2a and IgG2b were compared between untreated recipients and the recipients with accommodated heart graft. Sera from untreated recipients contained predominant IgG1 isotype, indicated by a high ratio of IgG1/IgG2a (Figure 5A). In contrast, a significant reduction in the ratio of IgG1/IgG2a was observed in the recipients carrying accommodated grafts (Figure 5A, $P<0.01$). Furthermore, presensitized recipients with the accommodated heart grafts displayed an increased level of anti-donor IgG2b as compared to the same recipients with rejected grafts (Figure 5B, $P<0.01$). In addition, the pattern of IgG isotypes in the recipients treated with either monotherapy or two drugs in combination is indistinguishable from that of untreated animals. These data indicate that anti-donor IgG1 isotype may be associated with graft rejection, while production of anti-donor IgG2b subclass may function as a protective antibody and plays an important role in the induction of accommodation.

Anti-C5 mAb in combination with CsA and CyP induces intragraft Bcl-2 and Bcl-xl expression in highly sensitized mouse recipients.

To determine whether a causal relationship exists between intragraft expression of protective proteins and graft resistance to humoral injury in this model, western blot analysis was employed to detect proteins of interest in heart graft tissues from highly sensitized mouse recipients. Long-term surviving heart grafts were found to express high levels of Bcl-2 and Bcl-xl proteins on POD100, and these proteins were detected as early as 12 days after heart transplantation in highly sensitized recipients receiving anti-C5 mAb-based triple therapy

Samsung Bioepis Exhibit No. 1002
Page 1312

(Figure 6). In contrast, there were no Bcl-2 and Bcl-xl proteins expressed on heart grafts of untreated animals (Figure 6) or animals treated with either monotherapy or two drugs in combination therapy. This result suggests that graft resistance to humoral injury in indefinite surviving animals is associated with the protection provided by Bcl-2 and Bcl-xl proteins in this presensitized model.

Presensitized recipients with an accommodating first heart graft accept a second accommodated heart graft but reject a second naive heart graft from the same donors.

The ability of accommodated grafts to resist rejection has not been tested directly under pathophysiological conditions where naive grafts undergo rejection following allotransplantation. In this model, to determine whether presensitized recipients with an accommodating first heart graft will accept a second accommodated graft but reject a second naive graft, we performed re-transplantation scenarios. After the accommodated C3H heart grafts have survived to the 100-day point, the time at which low levels of alloantibodies were detected (Figure 4B) and complement activity has returned to pretreatment levels (Figure 4D), in presensitized BALB/c recipient treated with anti-C5 mAb-based triple therapy, these recipients received a second heart graft. Specifically, either a naive (Figure 7A) or a 100-day accommodated C3H heart (Figure 7B) from another presensitized BALB/c recipient was transplanted into the neck of the presensitized recipients carrying an accommodating first C3H heart. These recipients rejected a second naive heart at 6.6 ± 1.1 days (Figure 8A) with severe AVR (Figure 8B-a) while the first heart continued to survive. In contrast, when the accommodated hearts that had been already surviving for 100 days in different presensitized mice were used as second grafts, these grafts were accepted by the presensitized recipients carrying an accommodating first heart graft (Figure 8A). There was no sign of rejection in those accommodated second heart grafts 90 days after second transplantation (Figure 8B-b). These data indicate that accommodated grafts become resistant to the effects of anti-donor antibodies and complement that normally mediate allograft rejection in these presensitized recipients. Furthermore, the fact that the host of the accommodated graft rejected a new graft suggests that accommodation involves changes to the graft.

Presensitized recipients being treated with CsA reject accommodated heart grafts.

Another re-transplantation was performed to determine whether accommodation in this presensitized model would be caused by the changes in the grafts and/or the recipients. Specifically, after C3H heart grafts have been accommodated in presensitized BALB/c mice

Samsung Bioepis Exhibit No. 1002
Page 1313

for 100 days, the accommodated heart graft will then be re-transplanted into a second presensitized BALB/c recipient being treated with CsA alone (Figure 7C), a therapy that can prevent cellular rejection but cannot prevent accelerated humoral rejection of a fresh C3H heart in presensitized recipients. The accommodated C3H heart grafts were rapidly rejected in CsA treated presensitized BALB/c recipients. After re-transplantation, the pathology in accommodated heart grafts was changed from normal (Figure 9A) to severe ACHR with massive interstitial hemorrhage but few cell infiltrates (Figure 9B). In addition, high levels of anti-donor IgG and normal levels of complement hemolytic activity in these recipients receiving an accommodated C3H heart were similar to those of CsA treated presensitized recipients receiving a naive C3H heart. This result further indicates that accommodation induced by anti-C5 mAb-based triple therapy can originate from mechanisms involving changes not only to the graft, but also to the recipient.

Example 3

Acute Vascular Rejection in a Heart Transplantation Model

Experiments were performed to determine whether inclusion of an inhibitor of formation of terminal complement would attenuate acute vascular rejection and whether the use of such an inhibitor in conjunction with an immunosuppressant would achieve long-term allograft survival. In this set of experiments an anti-C5 monoclonal antibody was used in conjunction with cyclosporin. The model used was an allograft heterotopic heart transplant from C3H mice into BALB/c mice. This model is a stringent acute vascular rejection model with the C3H and BALB/c mice being strongly MHC mismatched. The transplantations and other methods were performed as described in Wang et al. (2003).

Heterotopic Cardiac Transplantation

Intra-abdominal heterotopic cardiac transplantation was performed as previously described by Wang et al. (2003). Briefly, a median sternotomy was performed on the donor, and the heart graft was slowly perfused in situ with 1.0 ml of cold heparinized Ringer's lactate solution through the inferior vena cava and aorta before the superior vena cava and pulmonary veins were ligated and divided. The ascending aorta and pulmonary artery were transected, and the graft was removed from the donor. The graft was then revascularized with end-to-side anastomoses between the donor's pulmonary artery and the recipient's inferior vena cava as well as the donor's aorta and the recipient's abdominal aorta using 11-0 nylon suture. The beating of the grafted heart was monitored daily by direct abdominal palpation.

Samsung Bioepis Exhibit No. 1002
Page 1314

The degree of pulsation was scored as: A, beating strongly; B, noticeable decline in the intensity of pulsation; or C, complete cessation of cardiac impulses. When cardiac impulses were no longer palpable, the graft was removed for routine histology. In certain instances, mice in which the graft was still functioning were sacrificed to perform histology.

Results

Mice (male 8-12 week old mice weighing 25 – 30 g) were split into six experimental groups with six to eight mice per group. Transplant occurred on day 0. Histological changes were checked at the endpoint (the endpoint being graft failure) or in some cases a mouse was sacrificed prior to graft failure. The dosage of BB5.1 which was administered (40 mg/kg body weight three times per week) was known from prior studies to completely inhibit terminal complement activity.

Group 1' (control) - mice were administered 0.75 mL of saline intraperitoneally on days -1, 0, 1 and 2. Subsequently these mice were treated with 0.75 mL of saline intraperitoneally three times per week (Monday, Wednesday, Friday) until the endpoint.

Group 2' (cyclosporin A alone) - mice were administered 15 mg/kg body weight of cyclosporin A subcutaneously on a daily basis beginning at day 0 (day of transplant) until the endpoint.

Group 3' (anti-complement antibody alone) - mice were administered the anti-mouse C5 antibody BB5.1 (Frei et al., 1987) at 40 mg/kg body weight intraperitoneally on days -1, 0, 1 and 2 followed by 40 mg/kg body weight administered three times per week (Monday, Wednesday, Friday) until the endpoint.

Group 4' (anti-complement antibody until day 14 post-transplant plus cyclosporin A) - mice were administered the anti-mouse C5 antibody BB5.1 at 40 mg/kg body weight intravenously on days -1 through day 14 and were also administered cyclosporin A at 15 mg/kg of body weight on a daily basis beginning at day 0 until the endpoint. Note that this differs from the other groups in that the BB5.1 was administered intravenously and on a daily basis.

Group 5' (anti-complement antibody until day 28 post-transplant plus cyclosporin A) - mice were administered the anti-mouse C5 antibody BB5.1 at 40 mg/kg body weight intraperitoneally on days -1, 0, 1 and 2 followed by 40 mg/kg body weight administered three times per week (Monday, Wednesday, Friday) until day 28 and were also administered

Samsung Bioepis Exhibit No. 1002
Page 1315

cyclosporin A at 15 mg/kg of body weight on a daily basis beginning at day 0 until the endpoint.

Group 6' (anti-complement antibody chronically until 100 days plus cyclosporin) - mice were administered the anti-mouse C5 antibody BB5.1 at 40 mg/kg body weight intraperitoneally on days -1, 0, 1 and 2 followed by 40 mg/kg body weight administered three times per week (Monday, Wednesday, Friday) until 100 days and were also administered cyclosporin A at 15 mg/kg of body weight on a daily basis beginning at day 0 until 100 days.

The results of this experiment are shown in Tables 3 and 4. Table 3 shows the survival time for the grafts. Table 4 sets forth the histological scores.

Table 3

Allograft Survival

Group (Treatment)	Individual Survival (days)	Mean Survival Time (days)
1'. Saline	8, 8, 8, 8, 8, 9	8.3 ± 0.5
2'. Cyclosporin A	14, 15, 15, 16, 16, 16, 17	15.5 ± 1.1
3'. BB5.1	7, 8, 8, 8, 8, 9	8.0 ± 0.6
4'. BB5.1 until day 14 + cyclosporin A	35, 38, 43, 45, 46, 47	42.3 ± 4.8
5'. BB5.1 until day 28 + cyclosporin A	77, 80, 80, 81, 82	80 ±1.9
6'. BB5.1 until day 100 + cyclosporin A	>100 days (7 mice; one sacrificed for histology)	> 100 days

Samsung Bioepis Exhibit No. 1002
Page 1316

Table 4

Median Scores of Histological Changes of Heart Allografts at Necropsy

Groups	Vasc*	Infarc	Lymph	Throm	Hemo	Fibrin	PMN
1'. Saline (endpoint)	3.0	3.0	1.0	4.0	3.0	3.0	3.0
2'. Cyclosporin A (endpoint)	2.0	1.0	2.0	3.0	2.0	2.0	3.0
3'. BB5.1 (endpoint)	2.0	1.0	2.0	2.0	1.0	0.0	0.0
4'. BB5.1 until day 14 + cyclosporin A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
5'. BB5.1 28 days + Cyclosporin A (post-operative day 8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5'. BB5.1 28 days + Cyclosporin A (endpoint)	0.0	0.0	1.0	1.0	2.0	1.0	0.0
6'. BB5.1 until day 100 + Cyclosporin A (post-operative day 100)	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Median scores: 0 - normal; 1- minimum change; 2 - mild change; 3 - moderate change; 4 - marked change. N/A - not available.

*Vasc - vasculitis; Infar - infarction; Lymph - lymphocyte infiltration; Throm - thrombosis; Hemo - hemorrhage; Fibrin - fibrin deposition; PMN - polymorphonuclear cell infiltrate

The results indicate the synergistic effects of using a complement inhibiting drug in addition to an immunosuppressant. In untreated mice the grafts were rejected in about 8 davs. Use of the immunosuppressant cyclosporin A alone on a daily, chronic basis resulted

Samsung Bioepis Exhibit No. 1002
Page 1317

in an increase in graft survival until approximately 15 days post-transplant. The use of the anti-C5 antibody BB5.1 to inhibit formation of terminal complement had no effect on its own, graft rejection occurring at 8 days post-transplant as in the control group (Group 1'). The combination of BB5.1 through day 28 post-transplant plus cyclosporin A showed a synergistic effect with graft survival being extended until approximately day 80. A more surprising result is that of Group 5' in which BB5.1 and cyclosporin A were each administered chronically post-transplant. In this case the graft survival was for more than 100 days (as much data as presently available). Additionally, the histological results shown in Table 4 indicate that the administration of both BB5.1 and cyclosporin A protected the graft from changes much better than either BB5.1 or cyclosporin A alone, and that the chronic administration of BB5.1 and cyclosporin A protected the graft to such an extent that even at 100 days post-transplant there were no histological changes seen in the engrafted hearts. A survival time of 100 days in these models is considered to be the gold standard. A survival of 100 days in the model is believed to indicate that there will be an indefinite survival of the allograft. When BB5.1 administration was stopped after 28 days, the grafts were protected but they did begin to show some minimal to mild histological changes by about day 80 which was the time at which graft failure occurred.

The Group 4' mice were treated differently in that they were administered BB5.1 on a daily basis by an intravenous administration. These animals became ill, showing weight loss and urine retention and were sacrificed at a time at which the grafted hearts were still beating although their function had declined. This was the first group of mice studied and it is unknown why these ill effects were seen. These ill effects were not seen when the BB5.1 was administered intraperitoneally with a schedule of three times per week. As seen below in Example 4, daily administration of BB5.1 via an intraperitoneal route did not cause ill effects. Also, intravenous administration was not necessarily the cause of the illness in these animals. Intravenous administration of eculizumab (a human equivalent antibody to BB5.1 in that it binds to human C5) has been successfully administered intravenously without ill effects to humans in a study of PNH (Hillmen et al., 2004). Complement inhibitors may be administered by other routes in addition to intravenous and intraperitoneal, with all such routes being well known by those skilled in the art.

Example 4

Samsung Bioepis Exhibit No. 1002
Page 1318

Accelerated Rejection in a Presensitized Heart Transplantation Model

A second set of experiments similar to those of Example 3 was performed but the recipient mouse was presensitized to the donor organ. In these experiments, the presensitization was brought about by prior transplantation of a skin graft. In general, presensitization can occur not only as a result of having received an earlier allograft, but can also be caused by having received multiple blood transfusions or in women who have been pregnant. Besides such presensitization methods, allografts with an ABO mismatch will be rapidly attacked and rejected because of preformed antibodies to the ABO antigens unless steps are taken to prevent such an attack.

Some mice in these studies were administered cyclophosphamide in addition to BB5.1 and/or cyclosporin A. For these experiments BALB/c recipient mice were presensitized with C3H skin grafts one week prior to heart transplantation from the same donor (using the method of Pruitt and Bollinger, 1991). This model is designed to mimic presensitized transplantation in humans, especially in relation to accelerated humoral rejection. Recipient mice were split into eight groups of six to eight mice each. The treatments were as follow.

Group 1" (control) - mice (male 8-12 week old mice weighing 25 – 30 g) were administered 0.75 mL saline intraperitoneally on a daily basis beginning at day -1 and continuing until the endpoint (graft rejection).

Group 2" (cyclosporin A alone) - mice were administered cyclosporin A subcutaneously at a dose of 15 mg/kg body weight beginning on day 0 (day of transplant) until the endpoint.

Group 3" (BB5.1 alone) - mice were administered the anti-mouse complement monoclonal antibody BB5.1 at a dose of 40 mg/kg body weight delivered intraperitoneally on a daily basis beginning at day -1 and continuing until the endpoint.

Group 4" (cyclophosphamide alone) - mice were administered cyclophosphamide intravenously at a dose of 40 mg/kg body weight on each of days 0 and 1.

Group 5" (BB5.1 plus cyclosporin A) - mice were administered BB5.1 intraperitoneally at a dose of 40 mg/kg body weight on a daily basis beginning at day -1 and continuing until the endpoint. These mice were additionally administered cyclosporin A subcutaneously at a dose of 15 mg/kg body weight on a daily basis from day 0 until the endpoint.

Samsung Bioepis Exhibit No. 1002
Page 1319

Group 6" (BB5.1 plus cyclophosphamide) - mice were administered BB5.1 intraperitoneally at a dose of 40 mg/kg body weight on a daily basis beginning at day -1 and continuing until the endpoint. These mice were additionally administered cyclophosphamide intravenously at a dose of 40 mg/kg body weight on each of days 0 and 1.

Group 7" (cyclosporin A plus cyclophosphamide) - mice were administered cyclosporin A subcutaneously at a dose of 15 mg/kg body weight on a daily basis from day 0 until the endpoint. These mice were additionally administered cyclophosphamide intravenously at a dose of 40 mg/kg body weight on each of days 0 and 1.

Group 8" (BB5.1 plus cyclosporin A plus cyclophosphamide) - mice were administered BB5.1 intraperitoneally at a dose of 40 mg/kg body weight on a daily basis beginning at day -1 and continuing until 100 days. These mice were also administered cyclosporin A subcutaneously at a dose of 15 mg/kg body weight on a daily basis from day 0 until 100 days. These mice were additionally administered cyclophosphamide intravenously at a dose of 40 mg/kg body weight on each of days 0 and 1. Two mice in this group were sacrificed at day 60 for histological studies (no rejection had yet occurred) and the four remaining mice still had not rejected their grafts by day 100.

Additionally a control group of mice which was not presensitized and received only the saline treatment as for Group 1" was tested.

The results of these experiments are shown in Tables 5 and 6. Table 5 lists survival times for the grafts and Table 6 summarizes the histological results.

Samsung Bioepis Exhibit No. 1002
Page 1320

Table 5

Allograft Survival

Groups (Treatment)	Individual survival (days)	Mean survival time (days)
No presensitization	8, 8, 8, 8, 8, 9	8.3 ± 0.5*
1". One skin presensitization	3, 3, 3, 3, 3, 3, 3, 4	3.1 ± 0.4
2". Cyclosporin A	3, 3, 3, 3	3.0 ± 0.0
3". BB5.1	3, 3, 4, 4	3.5 ± 0.6
4". Cyclophosphamide	3, 3, 3, 4	3.3 ± 0.5
5". BB5.1 + Cyclosporin A	10, 10, 11, 11, 12, 12, 14, 15	11.9 ± 1.8**
6". BB5.1 + Cyclophosphamide	3, 3, 3, 3, 3, 4	3.2 ± 0.4
7". Cyclosporin A + Cyclophosphamide	3, 3, 3, 4, 4, 4	3.5 ± 0.6
8". BB5.1 + Cyclosporin A + Cyclophosphamide	> 100 days (4 mice)	> 100***

*P < 0.01 group 1" vs. no presensitization

**P < 0.01 group 5" vs. groups 1"-4" and 6"-7".

***P<0.01 group 8" vs. groups 1"-7".

Samsung Bioepis Exhibit No. 1002
Page 1321

Table 6

Median Scores of Histological Changes of Heart Allografts at Necropsy

Groups	Vasc*	Infar	Lymph	Throm	Hemo	Fibrin	PMN
No presensitization (endpoint)	3.0	3.0	1.0	4.0	3.0	3.0	3.0
1". One skin presensitization (endpoint)	0.0	4.0	1.0	4.0	3.0	0.0	0.0
2". Cyclosporin A (endpoint)	0.0	4.0	0.0	3.0	3.0	N/A	N/A
3". BB5.1 (endpoint)	2.0	2.0	2.0	2.0	3.0	N/A	N/A
4". Cyclophosphamide (endpoint)	2.0	4.0	0.0	3.0	2.0	N/A	N/A
5". BB5.1 + Cyclosporin A (post-operative day 3)	0.0	1.0	1.0	0.0	0.0	0.0	0.0
5". BB5.1 + Cyclosporin A (endpoint)	2.0	2.0	2.0	2.0	2.0	0.0	0.0
6". BB5.1 + Cyclophosphamide (endpoint)	1.0	3.0	0.0	3.0	2.0	N/A	N/A
7". Cyclosporin A + Cyclophosphamide (endpoint)	0.0	1.0	1.0	2.0	3.0	N/A	N/A
8". BB5.1 + Cyclosporin A + Cyclophosphamide (post-operative day 3)	0.0	0.0	1.0	0.0	0.0	N/A	N/A
8. BB5.1 + Cyclosporin A + Cyclophosphamide (post-operative day 12)	0.0	0.0	1.0	0.0	0.0	N/A	N/A
8". BB5.1 + Cyclosporin A + Cyclophosphamide (post-	0.0	0.0	1.0	0.0	0.0	N/A	N/A

Samsung Bioepis Exhibit No. 1002
Page 1322

operative day 60)							
8". BB5.1 + Cyclosporin A + Cyclophosphamide (post-operative day 100)	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Median scores: 0 - normal; 1- minimum change; 2 - mild change; 3 - moderate change; 4 - marked change. N/A - not available.

*Vasc - vasculitis; Infar - infarction; Lymph - lymphocyte infiltration; Throm - thrombosis; Hemo - hemorrhage; Fibrin - fibrin deposition; PMN - polymorphonuclear cell infiltrate

The results shown in Table 5 indicate a difference between the presensitized mouse model and the nonpresensitized mouse model as used in Example 3. The results indicate that in the absence of presensitization, grafts are rejected in approximately 8 days in the absence of treatment with any drugs. Presensitizing the animals causes a more rapid rejection, the rejection of the graft in the presensitized animals being in approximately 3 days in the absence of any drug treatment. Treatment with either BB5.1, cyclosporin A or cyclophosphamide had no effect upon graft survival, with the grafts being rejected in approximately 3-4 days in each of these groups of animals. The combination of BB5.1 and cyclosporin A showed some effect with rejection occurring about day 12. The combination of BB5.1 and cyclophosphamide had no protective effect with rejection occurring about day 3. Similarly the combination of cyclosporin A and cyclophosphamide had essentially no protective effect with rejection occurring at 3-4 days. Very surprisingly, the combination of all three drugs (chronic administration of BB5.1 and cyclosporin plus administration of cyclophosphamide at the time of transplant) showed a highly synergistic effect with all of the mice surviving for more than 100 days. Again, a survival of 100 days in this model is considered to be the gold standard and assumes an indefinite survival.

These results as well as the histological results as shown in Table 6 indicate that the combination of chronic treatment with a complement inhibitor and an immunosuppressant such as cyclosporin A in treating a presensitized mouse results in some attenuation of accelerated rejection. Treatment of these animals additionally with cyclophosphamide at the time of transplant and on the first day after transplant results in a much greater time of survival, no rejection having been seen by at least day 100.

Samsung Bioepis Exhibit No. 1002
Page 1323

It will be appreciated that the methods and compositions of the instant disclosure can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent to the artisan that other embodiments exist and do not depart from the spirit of the disclosure. Thus, the described embodiments are illustrative and should not be construed as restrictive.

Samsung Bioepis Exhibit No. 1002
Page 1324

LIST OF REFERENCES

The publications and other materials used herein to illuminate the background of the disclosure, and in particular, cases to provide additional details respecting the practice, are incorporated herein by reference in their entirety, and for convenience, are referenced by author and date in the text and respectively grouped in the following List of References.

Abbas AK, et al. (2000). Cellular and Molecular Immunology (4th edition), p. 363-383 (W.B. Saunders Company, New York).

Arp et al. (1996). *J. Virol.* 70:7349-7359.

Baldwin et al. (2001). *Immunity* 14:369-376.

Böhmig GA, et al. (2000). *Am. J. Kidney Dis.* 35:667-673.

Brauer et al. (1995). *Transplantation* 59:288-293.

Changelian PS, et al. (2003). *Science* 302:875-878.

Collard et al. (1997). Circulation 96:326-333.

Collins et al. (1999). *J. Am. Soc. Nephrol.* 10:2208-2214.

Fearon (1983). In Intensive Review of Internal Medicine, 2nd Ed. Fanta and Minaker, eds. Brigham and Women's and Beth Israel Hospitals.

Forbes et al. (1978). *Lab. Invest.* 39:463-470.

Frei Y, et al. (1987). *Mol. Cell. Probes* 1:141-149.

Gloor (2005). *Contrib. Nephrol.* 146:11-21.

Glotz et al. (1993). *Transplantation* 56:335-337.

Glotz D, et al. (2002). *Am. J. Transplant.* 2:758-760.

Hakim et al. (1990). *Am. J. Kidney Dis.* 16:423-431.

Halloran PF, et al. (1992). *Transplantation* 53:550-555.

Halloran (2003). *Am. J. Transplant.* 3:639-640.

Haviland DL, et al. (1991). *J. Immunol.* 146:362-368.

Hiesse C, et al. (1992). *Nephrol. Dial. Transplant.* 7:944-951.

Hillmen P, et al. (2004). *New Engl. J. Med.* 350:552-559.

Samsung Bioepis Exhibit No. 1002
Page 1325

Jeannet M, et al. (1970). *New Engl. J. Med.* 282:111-117.

Jones PT, et al. (1986). *Nature* 321:522-525.

Jose et al. (1983). *J. Exp. Med.* 158:2177-2182.

Kirschfink (2001). *Immunol. Rev.* 180:177-189.

Kobayashi et al. (1999). *J. Biol. Chem.* 274:28660-28668.

Kriaa et al. (1995). *Nephrol. Dial. Transplant. 10 Suppl.* 6:108-110.

Kroshus et al. (1995). *Transplantation* 60:1194-1202.

Kupiec-Weglinski (1996). *Ann. Transplant.* 1:34-40.

Kupin et al. (1991). *Transplantation* 51:324-329.

Liszewski (1993). Fundamental Immunology pp. 917-939.

Mauiyyedi et al. (2002). *Curr. Opin. Nephrol. Hypertens.* 11:609-618.

Mehra et al. (2003). *Curr. Opin. Cardiol.* 18:153-158.

Minta JO and Man DP (1977). *J. Immunol.* 119:1597-1602.

Montgomery RA, et al. (2000). *Transplantation* 70:887-895.

Olfert et al. (1993) Guide to the care and use of experimental animals (Vol.1). Ottawa: Association of Universities and Colleges of Canada 1.

Opelz G (1992). *Transplant. Proc.* 24:2342-2355.

OPTN/SRTR Annual Report (2002). Chapter 1 of the Annual Report produced by the Scientific Registry of Transplant Recipients (SRTR) in collaboration with the Organ Procurement and Transplantation Network (OPTN). See http://www.unos.org/data/ar2002/ar02_chapter_one.htm.

Palmer et al. (1989). *Lancet* 1:10-12.

Papadimitriou et al. (1991). *J. Immunol.* 147:212-217.

Park WD et al. (2003). *Am. J. Transplant* 3:952-960.

Persson NH et al. (1995). *Transplant. Proc.* 27:3466.

Platt et al. (1999). *Mol. Immunol.* 36:965-971.

Pratt et al. (1996). *Am J Pathol* 149:2055-2066.

Samsung Bioepis Exhibit No. 1002
Page 1326

Pratt et al. (2000). *Am. J. Pathol.* 157:825-831.

Pruitt et al. (1991). *J. Surg. Res.* 50:350-355.

Regele H, et al. (2001). *Nephrol. Dial. Transplant.* 16:2058-2066.

Rocha et al. (2003). *Transplantation* 75:1490-1495.

Ross et al. (1993). *Transplantation* 55:785-789.

Saadi et al. (1995). *J. Exp. Med.* 182:1807-1814.

Salama et al. (2001). *Am. J. Transplant.* 1:260-269.

Schweitzer et al. (2000). *Transplantation* 70:1531-1536.

Sonnenday et al. (2002). *Transplant. Proc.* 34:1614-1616.

Stepkowski SM (2000). *Exp. Rev. Mol. Med.* 21 June, http://www-ermm.cbcu.cam.ac.uk /00001769h.htm.

Taube DH, et al. (1984). *Lancet* 1:824-828.

Tyan et al. (1994). *Transplantation* 57:553-562.

Vakeva et al. (1998). *Circulation* 97:2259-2267.

Vogt W, et al. (1989). *Molec. Immunol.* 26:1133-1142.

Wang et al. (1995). *Proc. Natl. Acad. Sci. U. S. A.* 92:8955-8959.

Wang et al. (1996). *Proc. Natl. Acad. Sci. U. S. A.* 93:8563-8568.

Wang et al. (1999). *Transplantation* 68:1643-1651.

Wang H, et al. (2003). *J. Immunol.* 171:3823-3836.

Warren et al. (2004). *Am. J. Transplant.* 4:561-568.

Wetsel RA and Kolb WP (1982). *J. Immunol.* 128:2209-2216.

Wurzner R, et al. (1991). *Complement Inflamm.* 8:328-340.

Samsung Bioepis Exhibit No. 1002
Page 1327

Yamamoto KI and Gewurz G (1978). *J. Immunol.* 120:2008-2015.

PCT Patent Application Publication WO 92/10205

PCT Patent Application Publication WO 00/27421

PCT Patent Application Publication WO 01/37860

U.S. Patent Application Publication US 2003/0129187

U.S. Patent Application Publication US 2003/0180301

U.S. Patent 5,133,916

U.S. Patent 5,573,940

U.S. Patent 5,660,825

U.S. Patent 6,057,131

U.S. Patent 6,100,443

U.S. Patent 6,192,891

U.S. Patent 6,280,957

U.S. Patent 6,302,855

U.S. Patent 6,355,245

U.S. Patent 6,534,058

U.S. Patent 6,821,515

The contents of all references cited herein are incorporated by reference in their entirety.

Samsung Bioepis Exhibit No. 1002
Page 1328

CLAIMS

1. A method to prolong survival of an MHC mismatched allograft in a recipient mammal, said method comprising administering to said mammal a) a drug which inhibits complement activity and b) at least one immunosuppressive drug, wherein said drug which inhibits complement activity is administered chronically.

2. The method of claim 1 wherein said mammal is a human.

3. The method of claim 2 wherein said MHC mismatched allograft is an HLA mismatched allograft.

4. The method of claim 1 wherein said drug which inhibits complement activity inhibits the formation of terminal complement or C5a.

5. The method of claim 4 wherein said drug which inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment.

6. The method of claim 5 wherein said whole antibody or antibody fragment is a human, humanized, chimerized or deimmunized antibody or antibody fragment.

7. The method of claim 5 wherein said whole antibody or antibody fragment inhibits cleavage of complement C5.

8. The method of claim 5 wherein said antibody fragment is selected from the group consisting of an Fab, an $F(ab')_2$, an Fv, a domain antibody, and a single-chain antibody.

9. The method of claim 5 wherein said antibody fragment is pexelizumab.

10. The method of claim 5 wherein said whole antibody is eculizumab.

11. The method claim 10 wherein said eculizumab is administered once every 2 weeks.

12. The method of claim 1 wherein said inhibitor of complement activity is selected from the group consisting of a i) soluble complement receptor, ii) CD59, iii) CD55, iv) CD46, and v) an antibody to C5, C6, C7, C8, or C9.

13. The method of claim 1 wherein said immunosuppressive drug inhibits T-cell activity or B-cell activity.

Samsung Bioepis Exhibit No. 1002
Page 1329

14. The method of claim 1 wherein said immunosuppressive drug inhibits T-cell activity and B-cell activity.

15. The method of claim 1 wherein said immunosuppressive drug is selected from the group consisting of cyclosporin A, tacrolimus, sirolimus, OKT3, a corticosteroid, daclizumab, basiliximab, azathioprene, mycophenolate mofetil, methotrexate, 6-mercaptopurine, anti-T cell antibodies, cyclophosphamide, leflunamide, brequinar, ATG, ALG, 15-deoxyspergualin, and bredinin.

16. The method of claim 1 wherein more than one immunosuppressive drug is administered.

17. The method of claim 1 wherein said method comprises administering i) a drug which inhibits complement activity and ii) cyclosporin A.

18. The method of claim 17 wherein said drug which inhibits complement activity is an antibody which inhibits cleavage of complement C5.

19. The method of claim 1 wherein said allograft is selected from the group consisting of i) heart, ii) kidney, iii) lung, iv) pancreas, v) liver, vi) vascular tissue, vii) eye, viii) cornea, ix) lens, x) skin, xi) bone marrow, xii) muscle, xiii) connective tissue, xiv) gastrointestinal tissue, xv) nervous tissue, xvi) bone, xvii) stem cells, xviii) islets, xix) cartilage, xx) hepatocytes, and xxi) hematopoietic cells.

20. The method of claim 1 wherein said allograft survives for a time at least 20% longer than would occur if said method were to be performed without said drug which inhibits complement activity.

21. The method of claim 1 wherein said allograft survives for a time at least 40% longer than would occur if said method were to be performed without said drug which inhibits complement activity.

22. The method of claim 1 wherein said allograft survives for the remaining life-time of said mammal.

23. The method of claim 2 wherein said allograft survives for at least six months.

24. The method of claim 2 wherein said allograft survives for at least one year.

25. The method of claim 2 wherein said allograft survives for at least five years.

Samsung Bioepis Exhibit No. 1002
Page 1330

26. The method of claim 2 wherein said allograft survives for the remaining life-time of said human.

27. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 14 days.

28. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 28 days.

29. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 3 months.

30. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 6 months.

31. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 1 year.

32. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 5 years.

33. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for the remaining life-time of said mammal.

34. The method of claim 1 wherein at least one immunosuppressive drug is administered chronically for the remaining life-time of said mammal.

35. The method of claim 34 wherein at least cyclosporin A is administered chronically for the remaining life-time of said mammal.

36. A method to prolong survival of an allograft in a recipient mammal presensitized to said allograft, said method comprising administering to said mammal a) a drug which inhibits complement activity and b) at least one immunosuppressive drug, wherein said drug which inhibits complement activity is administered chronically.

37. The method of claim 36 wherein said mammal is a human.

38. The method of claim 36 wherein said drug which inhibits complement activity inhibits the formation of terminal complement or C5a.

Samsung Bioepis Exhibit No. 1002
Page 1331

39. The method of claim 38 wherein said drug that inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment.

40. The method of claim 39 wherein said whole antibody or antibody fragment is a human, humanized, chimerized or deimmunized antibody or antibody fragment.

41. The method of claim 39 wherein said whole antibody or antibody fragment inhibits cleavage of complement C5.

42. The method of claim 39 wherein said antibody fragment is selected from the group consisting of an Fab, an $F(ab')_2$, an Fv, a domain antibody, and a single-chain antibody.

43. The method of claim 39 wherein said antibody fragment is pexelizumab.

44. The method of claim 39 wherein said whole antibody is eculizumab.

45. The method claim 44 wherein said eculizumab is administered once every 2 weeks.

46. The method of claim 36 wherein said inhibitor of complement activity is selected from the group consisting of a i) soluble complement receptor, ii) CD59, iii) CD55, iv) CD46, and v) an antibody to C5, C6, C7, C8, or C9.

47. The method of claim 36 wherein said immunosuppressive drug inhibits T-cell activity or B-cell activity.

48. The method of claim 36 wherein said immunosuppressive drug inhibits T-cell activity and B-cell activity.

49. The method of claim 36 wherein said immunosuppressive drug is selected from the group consisting of cyclosporin A, tacrolimus, sirolimus, OKT3, a corticosteroid, daclizumab, basiliximab, azathioprene, mycophenolate mofetil, methotrexate, 6-mercaptopurine, anti-T cell antibodies, cyclophosphamide, leflunamide, brequinar, ATG, ALG, 15-deoxyspergualin, and bredinin.

50. The method of claim 36 wherein more than one immunosuppressive drug is administered.

51. The method of claim 36 wherein said method comprises administering i) a drug which inhibits complement activity and ii) cyclosporin A.

Samsung Bioepis Exhibit No. 1002
Page 1332

52. The method of claim 51 wherein said method comprises administering i) a drug which inhibits complement activity, ii) cyclosporin A, and iii) cyclophosphamide.

53. The method of claim 51 wherein said drug which inhibits complement activity is an antibody which inhibits cleavage of complement C5.

54. The method of claim 36 wherein said allograft is selected from the group consisting of i) heart, ii) kidney, iii) lung, iv) pancreas, v) liver, vi) vascular tissue, vii) eye, viii) cornea, ix) lens, x) skin, xi) bone marrow, xii) muscle, xiii) connective tissue, xiv) gastrointestinal tissue, xv) nervous tissue, xvi) bone, xvii) stem cells, xviii) islets, xix) cartilage, xx) hepatocytes, and xxi) hematopoietic cells.

55. The method of claim 36 wherein said allograft survives for a time at least 20% longer than would occur if said method were to be performed without said drug which inhibits complement activity.

56. The method of claim 36 wherein said allograft survives for a time at least 40% longer than would occur if said method were to be performed without said drug which inhibits complement activity.

57. The method of claim 36 wherein said allograft survives for the remaining life-time of said mammal.

58. The method of claim 37 wherein said allograft survives for at least six months.

59. The method of claim 37 wherein said allograft survives for at least one year.

60. The method of claim 37 wherein said allograft survives for at least five years.

61. The method of claim 37 wherein said allograft survives for the remaining life-time of said human.

62. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 14 days.

63. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 28 days.

64. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 3 months.

Samsung Bioepis Exhibit No. 1002
Page 1333

65. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 6 months.

66. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 1 year.

67. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 5 years.

68. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for the remaining life-time of said mammal.

69. The method of claim 36 wherein at least one immunosuppressive drug is administered chronically for the remaining life-time of said mammal.

70. The method of claim 69 wherein at least cyclosporin A is administered chronically for the remaining life-time of said mammal.

71. A method to prolong survival of an allograft in a recipient mammal which is an ABO mismatch to said allograft, said method comprising administering to said mammal a) a drug which inhibits complement activity and b) at least one immunosuppressive drug, wherein said drug which inhibits complement activity is administered chronically.

72. The method of claim 71 wherein said mammal is a human.

73. The method of claim 71 wherein said drug which inhibits complement activity inhibits the formation of terminal complement or C5a.

74. The method of claim 73 wherein said drug which inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment.

75. The method of claim 74 wherein said whole antibody or antibody fragment is a human, humanized, chimerized or deimmunized antibody or antibody fragment.

76. The method of claim 74 wherein said whole antibody or antibody fragment inhibits cleavage of complement C5.

77. The method of claim 74 wherein said antibody fragment is selected from the group consisting of an Fab, an $F(ab')_2$, an Fv, a domain antibody, and a single-chain antibody.

Samsung Bioepis Exhibit No. 1002
Page 1334

78. The method of claim 74 wherein said antibody fragment is pexelizumab.

79. The method of claim 74 wherein said whole antibody is eculizumab.

80. The method claim 79 wherein said eculizumab is administered once every 2 weeks.

81. The method of claim 71 wherein said inhibitor of complement activity is selected from the group consisting of a i) soluble complement receptor, ii) CD59, iii) CD55, iv) CD46, and v) an antibody to C5, C6, C7, C8, or C9.

82. The method of claim 71 wherein said immunosuppressive drug inhibits T-cell activity or B-cell activity.

83. The method of claim 71 wherein said immunosuppressive drug inhibits T-cell activity and B-cell activity.

84. The method of claim 71 wherein said immunosuppressive drug is selected from the group consisting of cyclosporin A, tacrolimus, sirolimus, OKT3, a corticosteroid, daclizumab, basiliximab, azathioprene, mycophenolate mofetil, methotrexate, 6-mercaptopurine, anti-T cell antibodies, cyclophosphamide, leflunamide, brequinar, ATG, ALG, 15-deoxyspergualin, and bredinin.

85. The method of claim 71 wherein more than one immunosuppressive drug is administered.

86. The method of claim 71 wherein said method comprises administering i) a drug which inhibits complement activity and ii) cyclosporin A.

87. The method of claim 86 wherein said method comprises administering i) a drug which inhibits complement activity, ii) cyclosporin A, and iii) cyclophosphamide.

88. The method of claim 86 wherein said drug that inhibits complement activity is an antibody that inhibits cleavage of complement C5.

89. The method of claim 71 wherein said allograft is selected from the group consisting of i) heart, ii) kidney, iii) lung, iv) pancreas, v) liver, vi) vascular tissue, vii) eye, viii) cornea, ix) lens, x) skin, xi) bone marrow, xii) muscle, xiii) connective tissue, xiv) gastrointestinal tissue, xv) nervous tissue, xvi) bone, xvii) stem cells, xviii) islets, xix) cartilage, xx) hepatocytes, and xxi) hematopoietic cells.

Samsung Bioepis Exhibit No. 1002
Page 1335

90. The method of claim 71 wherein said allograft survives for a time at least 20% longer than would occur if said method were to be performed without said drug that inhibits complement activity.

91. The method of claim 71 wherein said allograft survives for a time at least 40% longer than would occur if said method were to be performed without said drug that inhibits complement activity.

92. The method of claim 71 wherein said allograft survives for the remaining life-time of said mammal.

93. The method of claim 72 wherein said allograft survives for at least six months.

94. The method of claim 72 wherein said allograft survives for at least one year.

95. The method of claim 72 wherein said allograft survives for at least five years.

96. The method of claim 72 wherein said allograft survives for the remaining life-time of said human.

97. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 14 days.

98. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 28 days.

99. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 3 months.

100. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 6 months.

101. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 1 year.

102. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 5 years.

103. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for the remaining life-time of said mammal.

Samsung Bioepis Exhibit No. 1002
Page 1336

104. The method of claim 71 wherein at least one immunosuppressive drug is administered chronically for the remaining life-time of said mammal.

105. The method of claim 104 wherein at least cyclosporin A is administered chronically for the remaining life-time of said mammal.

106. Use of a drug that inhibits complement activity and an immunosuppressive drug in the manufacture of a medicament or medicament package for prolong survival of an allograft in a mammal.

107. The use of claim 106, wherein more than one immunosuppressive drug is included in said medicament or medicament package.

108. The use of claim 106, wherein said drug that inhibits complement activity and said immunosuppressive drug are in a formulation suitable for concurrent administration to said mammal.

109. The use of claim 106, wherein said drug that inhibits complement activity and said immunosuppressive drug are in a formulation or formulations suitable for sequential administration to said mammal.

110. The use of claim 106, wherein said drug that inhibits complement activity is in a formulation suitable for chronic administration to said mammal.

111. The use of claim 106, wherein said immunosuppressive drug is in a formulation suitable for chronic administration to said mammal.

112. A method to prolong survival of allograft in a recipient mammal, comprising preparing an allograft from a first allograft accommodated by a first recipient mammal, wherein said first recipient mammal, after receiving said first allograft, has been treated with a drug that inhibits complement activity and at least one immunosuppressive agent.

113. An allograft that has prolonged survival in a recipient mammal, wherein said allograft is prepared from a first recipient mammal and was transplanted to the first recipient mammal that has been treated with a drug that inhibits complement activity and at least one immunosuppressive agent.

Samsung Bioepis Exhibit No. 1002
Page 1337

114. A pharmaceutical package comprising a drug that inhibits complement activity and at least one immunosuppressive agent, wherein said drug and said agent are formulated for chronic administration.

115. The pharmaceutical package of claim 114, wherein said drug is an antibody in a lyophilized formulation comprising the antibody and a lyoprotectant.

116. The pharmaceutical package of claim 114, wherein said agent is in a lyophilized formulation comprising the agent and a lyoprotectant.

117. The pharmaceutical package of claim 114, wherein said drug and said agent are in the same lyophilized formulation comprising said drug, said agent, and a lyoprotectant.

118. The pharmaceutical package of claim 114, wherein said drug is in an injection system comprising a syringe that comprises a cartridge, wherein said cartridge contains said drug in a formulation suitable for injection.

119. The pharmaceutical package of claim 114, wherein said agent is in an injection system comprising a syringe that comprises a cartridge, wherein said cartridge contains said agent in a formulation suitable for injection.

120. The pharmaceutical package of claim 114, wherein said drug and said agent are in an injection system comprising a syringe that comprises a cartridge, wherein said cartridge contains said drug and said agent in a formulation suitable for injection.

121. The pharmaceutical package of claim 114, wherein said drug is present in unit dosage form.

122. The pharmaceutical package of claim 114, wherein said agent is present in unit dosage form.

123. A lyophilized antibody formulation comprising an antibody that inhibits complement activity and a lyoprotectant, wherein said antibody formulation is suitable for chronic administration.

124. The lyophilized antibody formulation of claim 123, wherein the antibody inhibits the formation of terminal complement or C5a.

Samsung Bioepis Exhibit No. 1002
Page 1338

125. The lyophilized antibody formulation of claim 123, wherein the antibody is a whole antibody or an antibody fragment.

126. The lyophilized antibody formulation of claim 125, wherein the antibody wherein said whole antibody or antibody fragment is a human, humanized, chimerized or deimmunized antibody or antibody fragment.

127. The lyophilized antibody formulation of claim 125, wherein said whole antibody or antibody fragment inhibits cleavage of complement C5.

128. The lyophilized antibody formulation of claim 125, wherein said antibody fragment is selected from the group consisting of an Fab, an $F(ab')_2$, an Fv, a domain antibody, and a single-chain antibody.

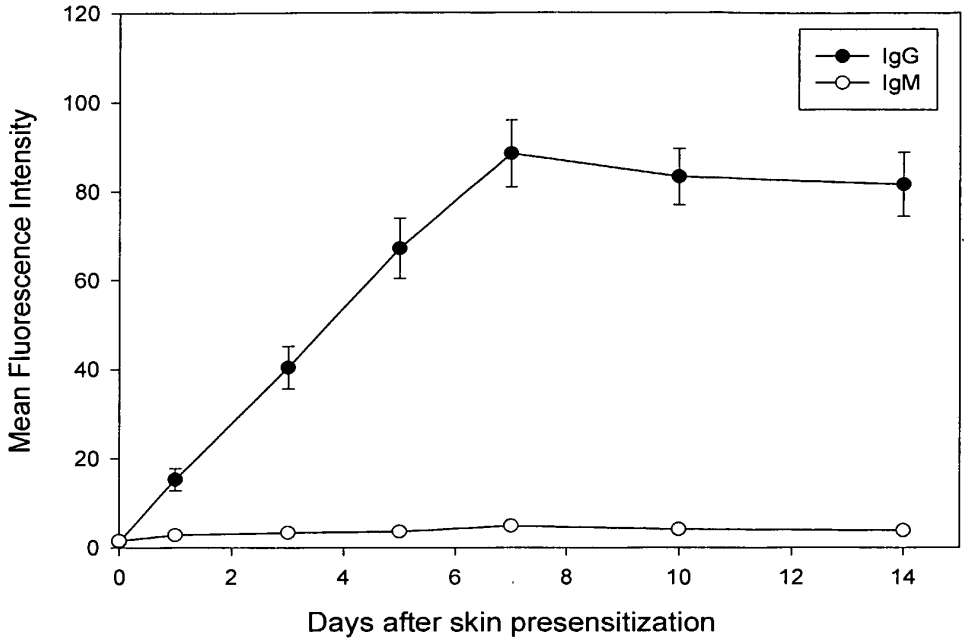
129. The lyophilized antibody formulation of claim 125, wherein said antibody fragment is pexelizumab.

130. The lyophilized antibody formulation of claim 125, wherein said whole antibody is eculizumab.

131. An injection system comprising a syringe, wherein said syringe comprises a cartridge containing an antibody that inhibits complement activity and is formulated suitable for injection.

Samsung Bioepis Exhibit No. 1002
Page 1339

A



B

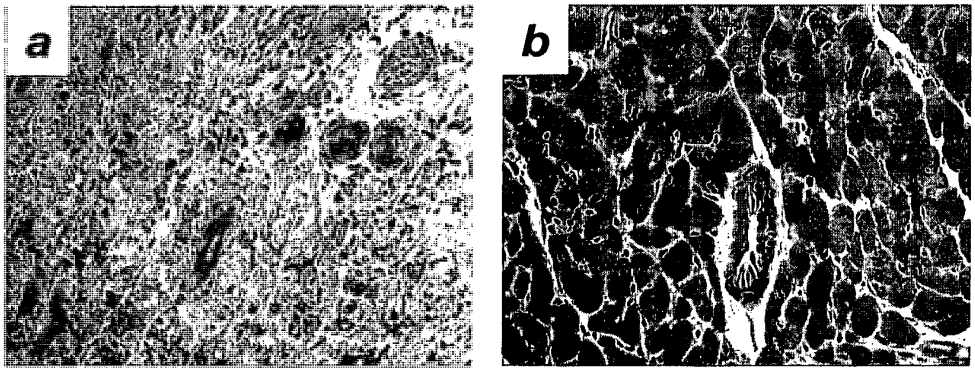


Figure 1A, B

Samsung Bioepis Exhibit No. 1002
Page 1340

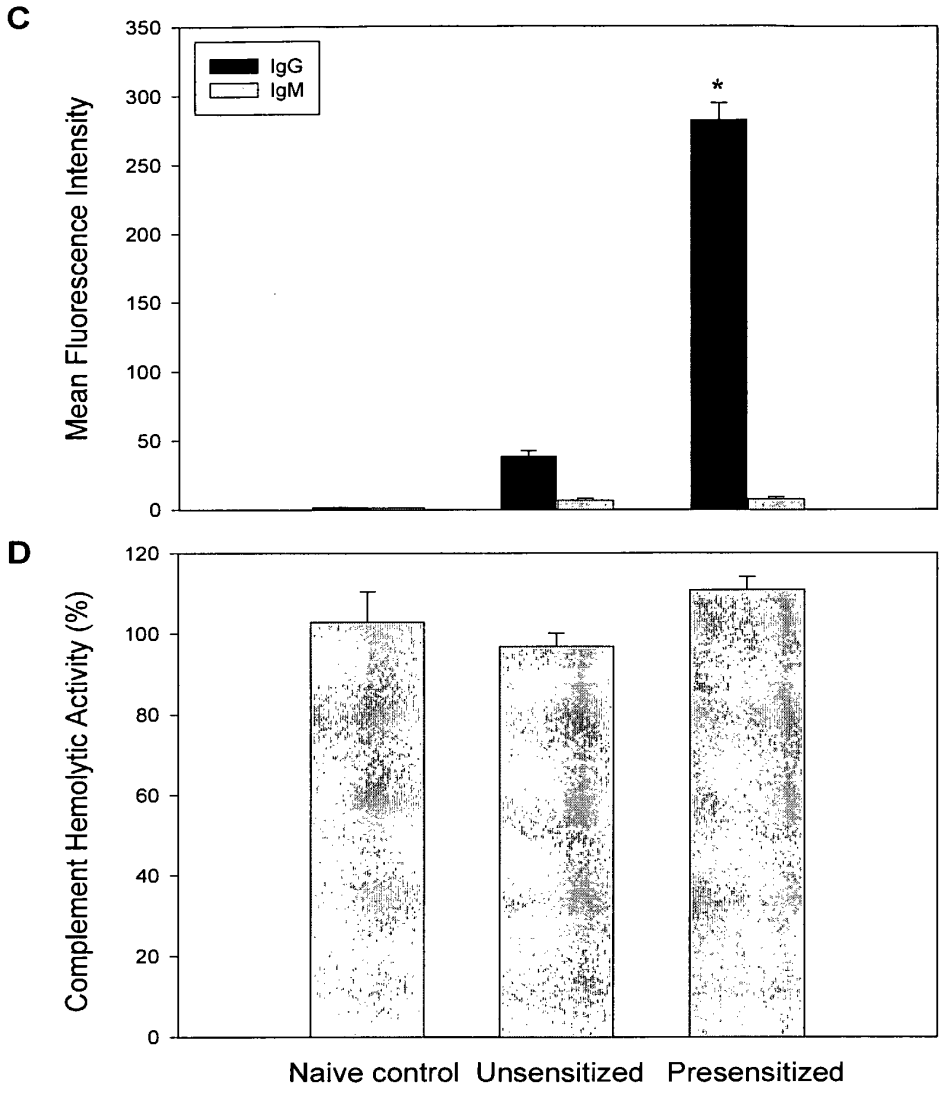


Figure 1C, D

Samsung Bioepis Exhibit No. 1002
Page 1341

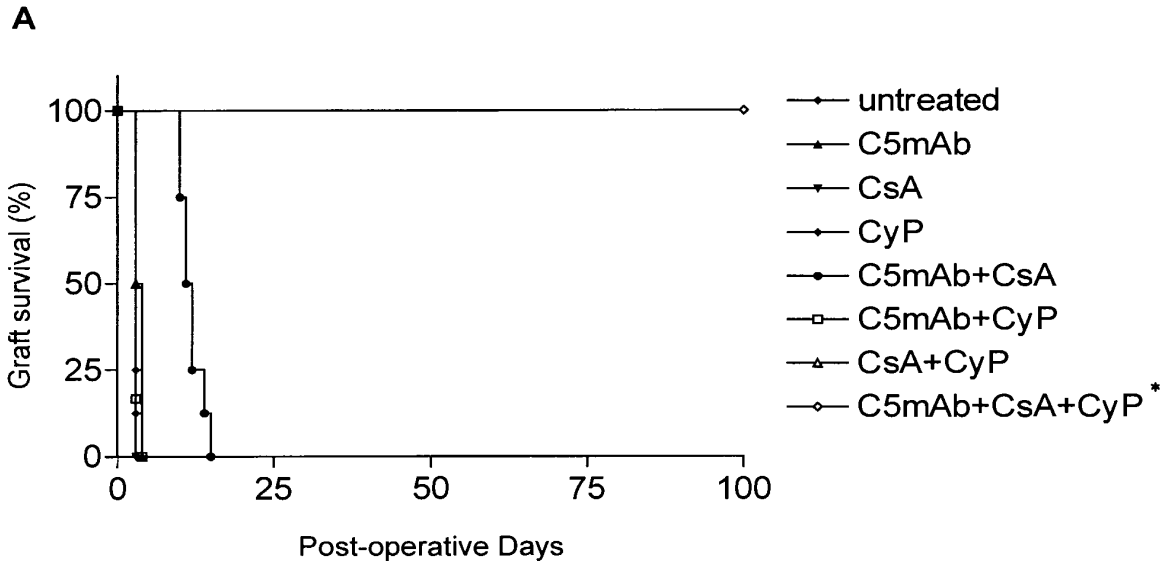


Figure 2A

Samsung Bioepis Exhibit No. 1002
Page 1342

B

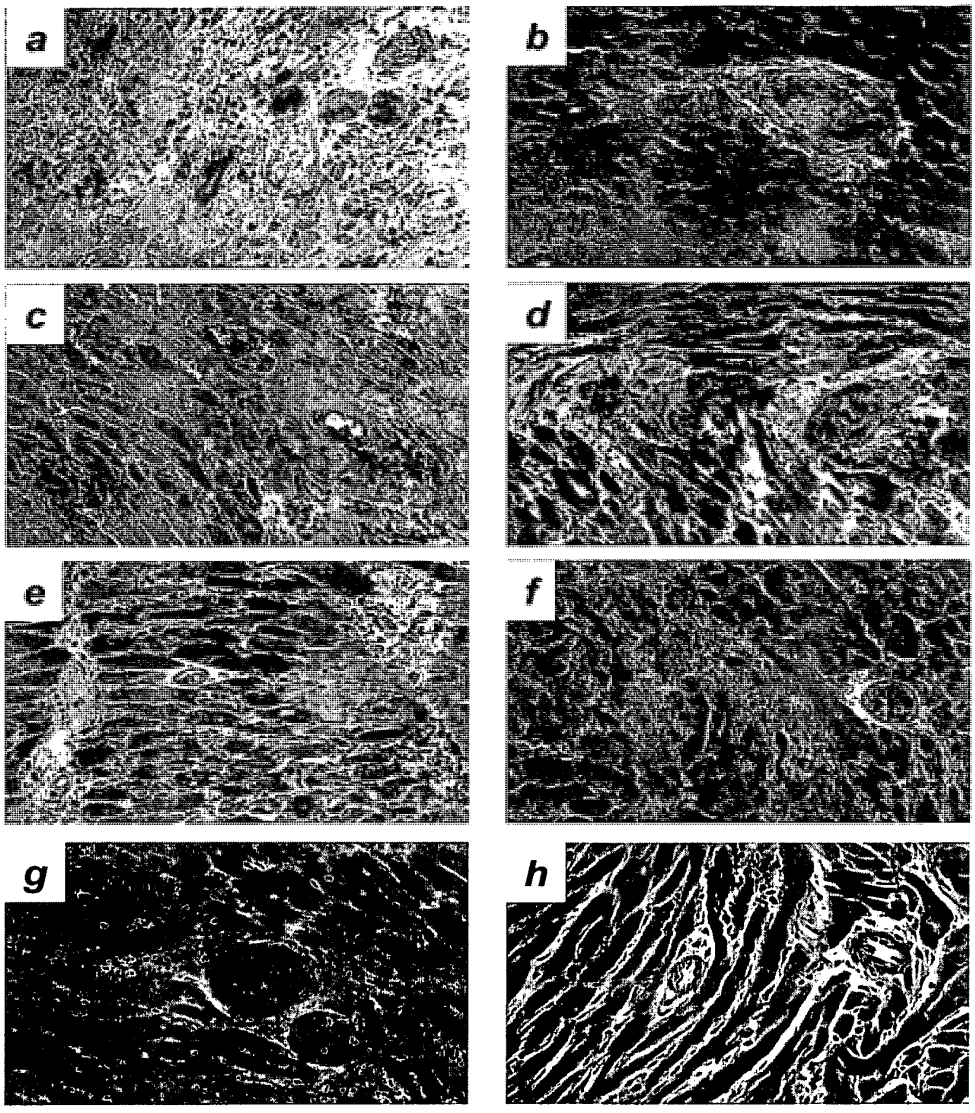


Figure 2B

Samsung Bioepis Exhibit No. 1002
Page 1343

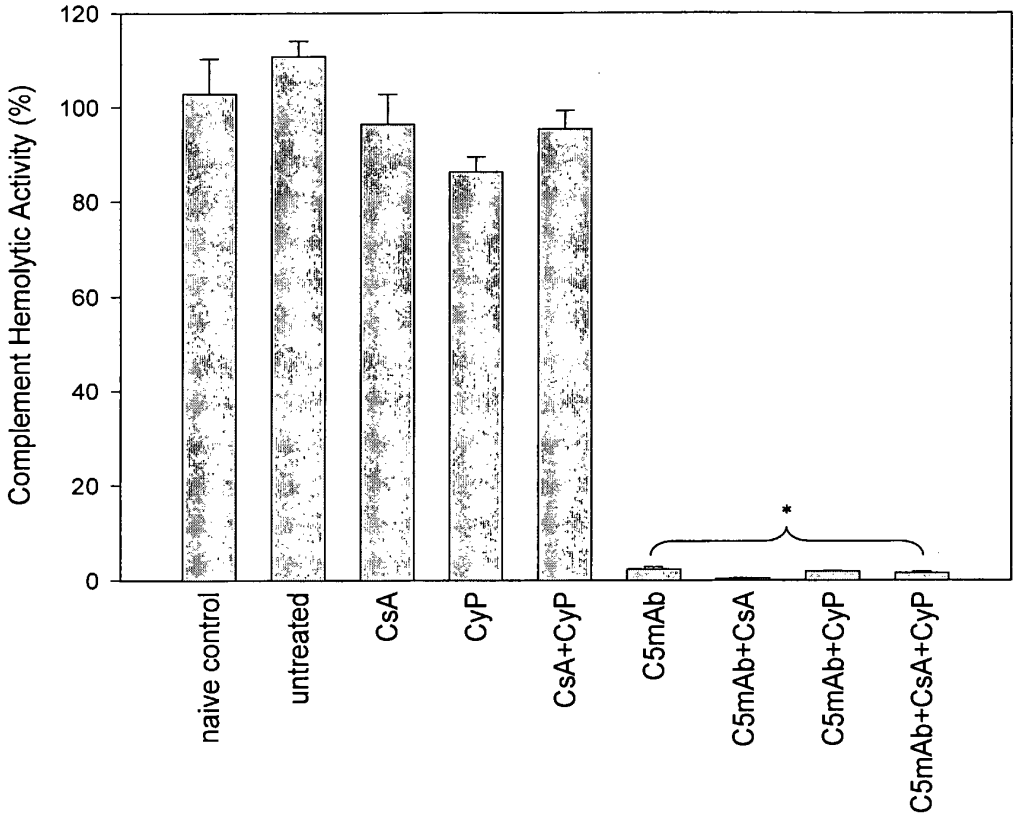


Figure 3

Samsung Bioepis Exhibit No. 1002
Page 1344

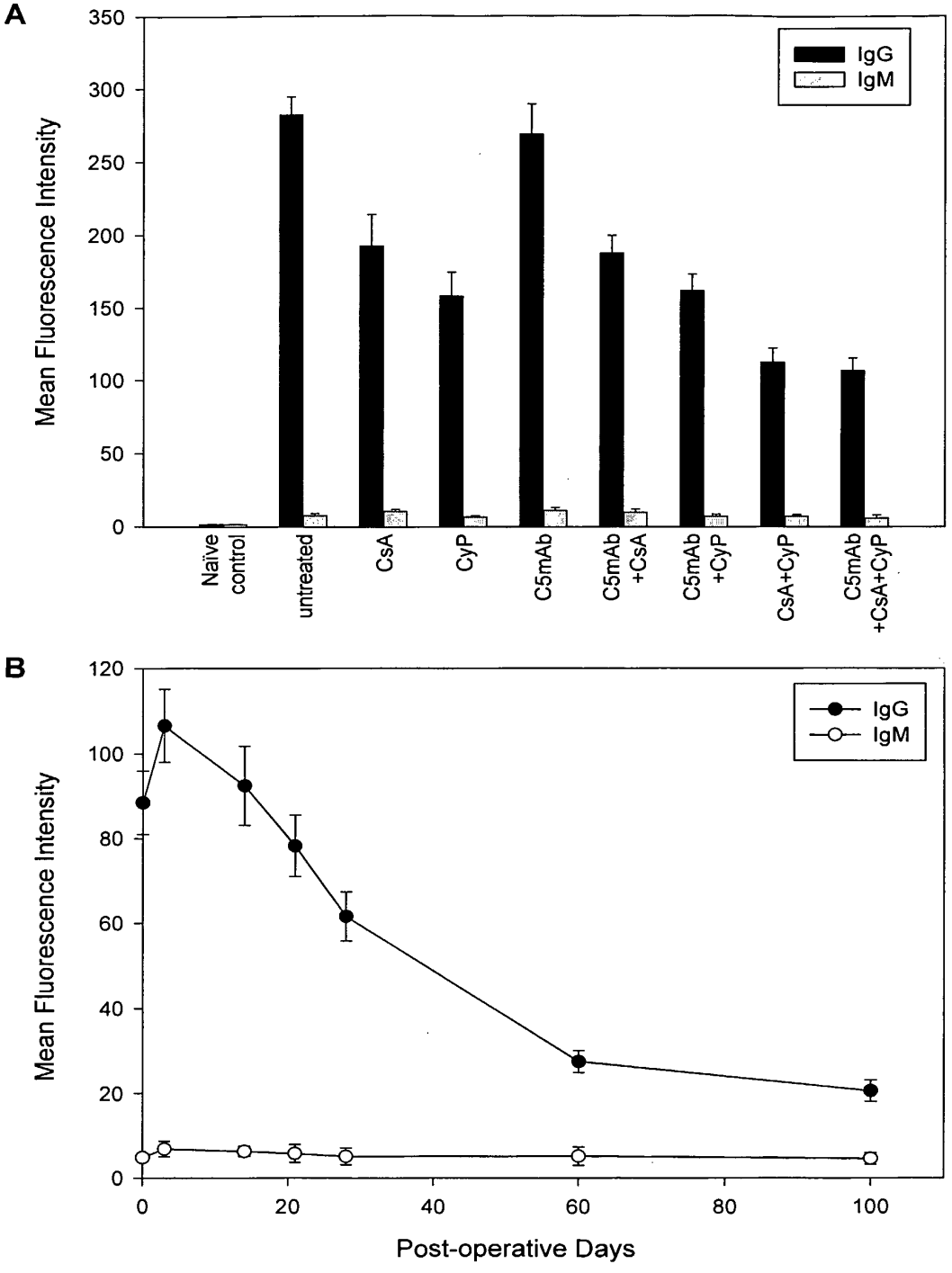
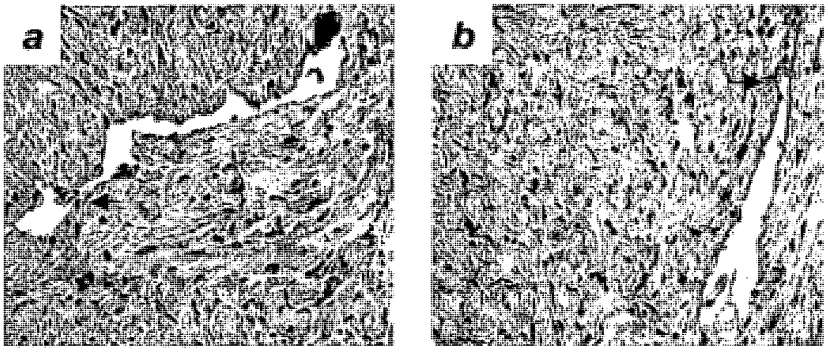


Figure 4A, B

Samsung Bioepis Exhibit No. 1002
Page 1345

C



D

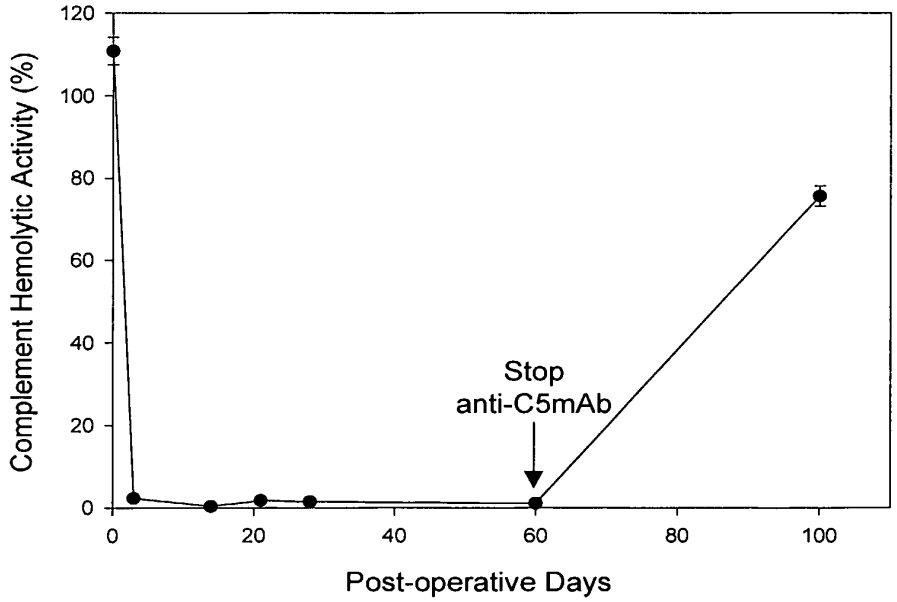


Figure 4C, D

Samsung Bioepis Exhibit No. 1002
Page 1346

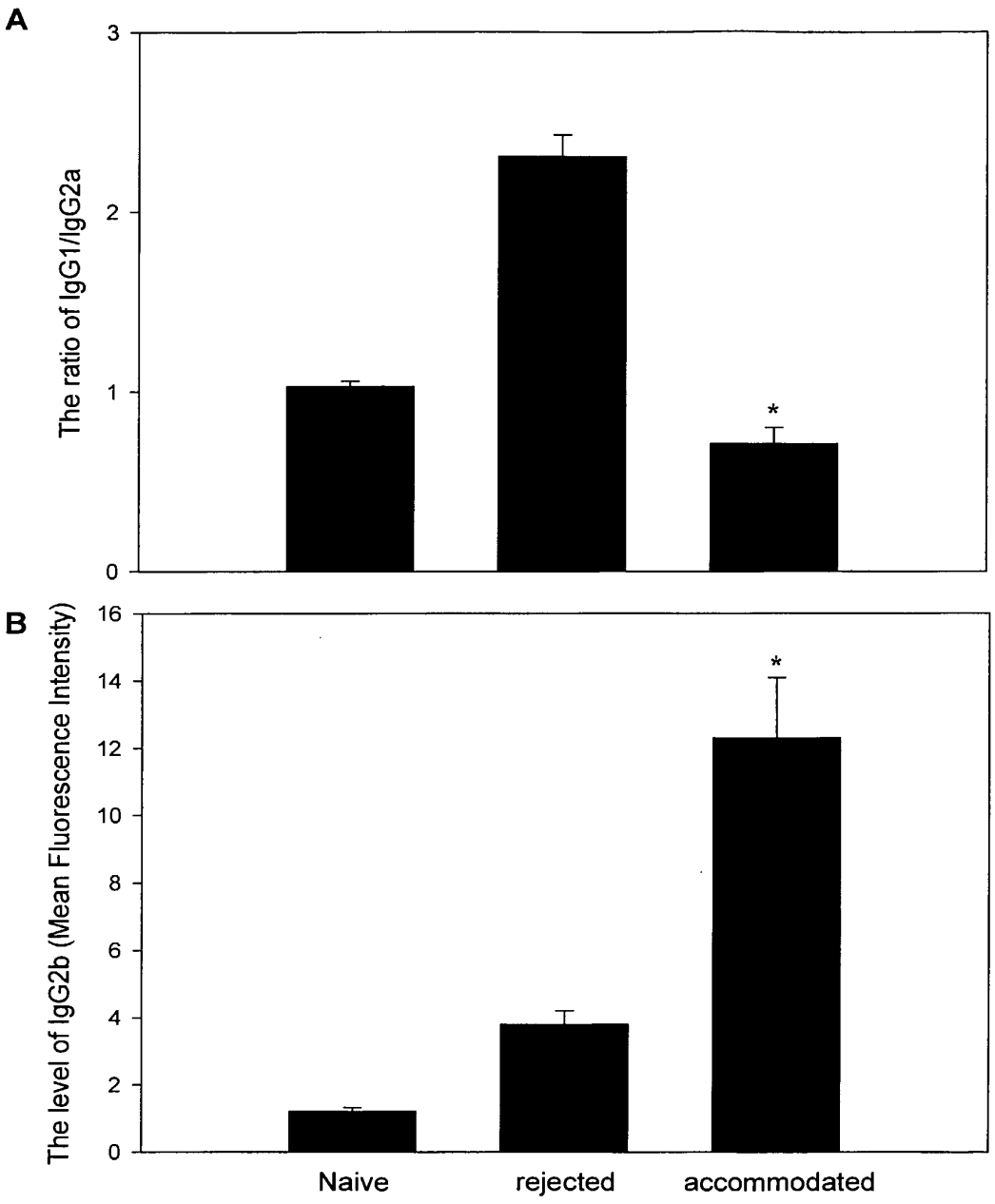


Figure 5A, B

Samsung Bioepis Exhibit No. 1002
Page 1347

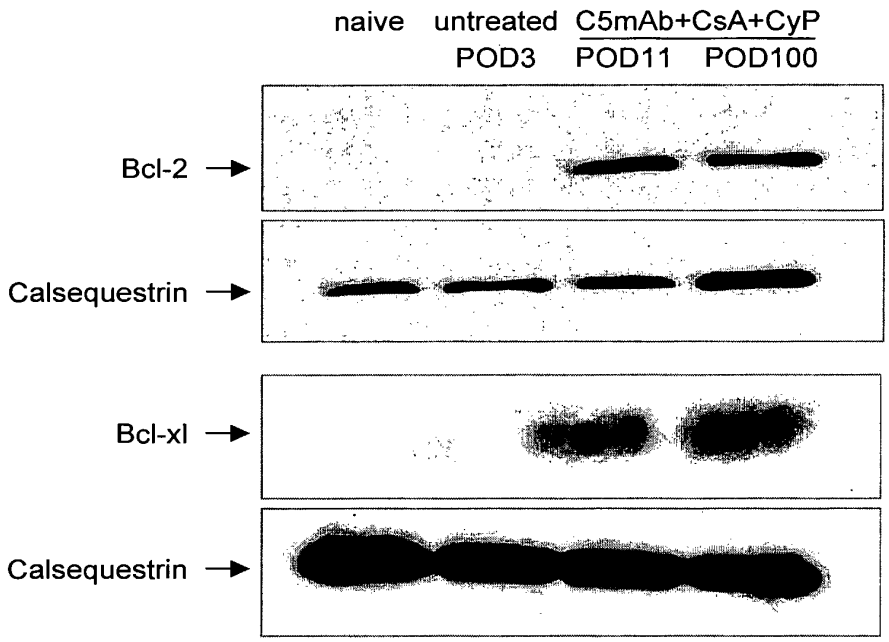


Figure 6

Samsung Bioepis Exhibit No. 1002
Page 1348

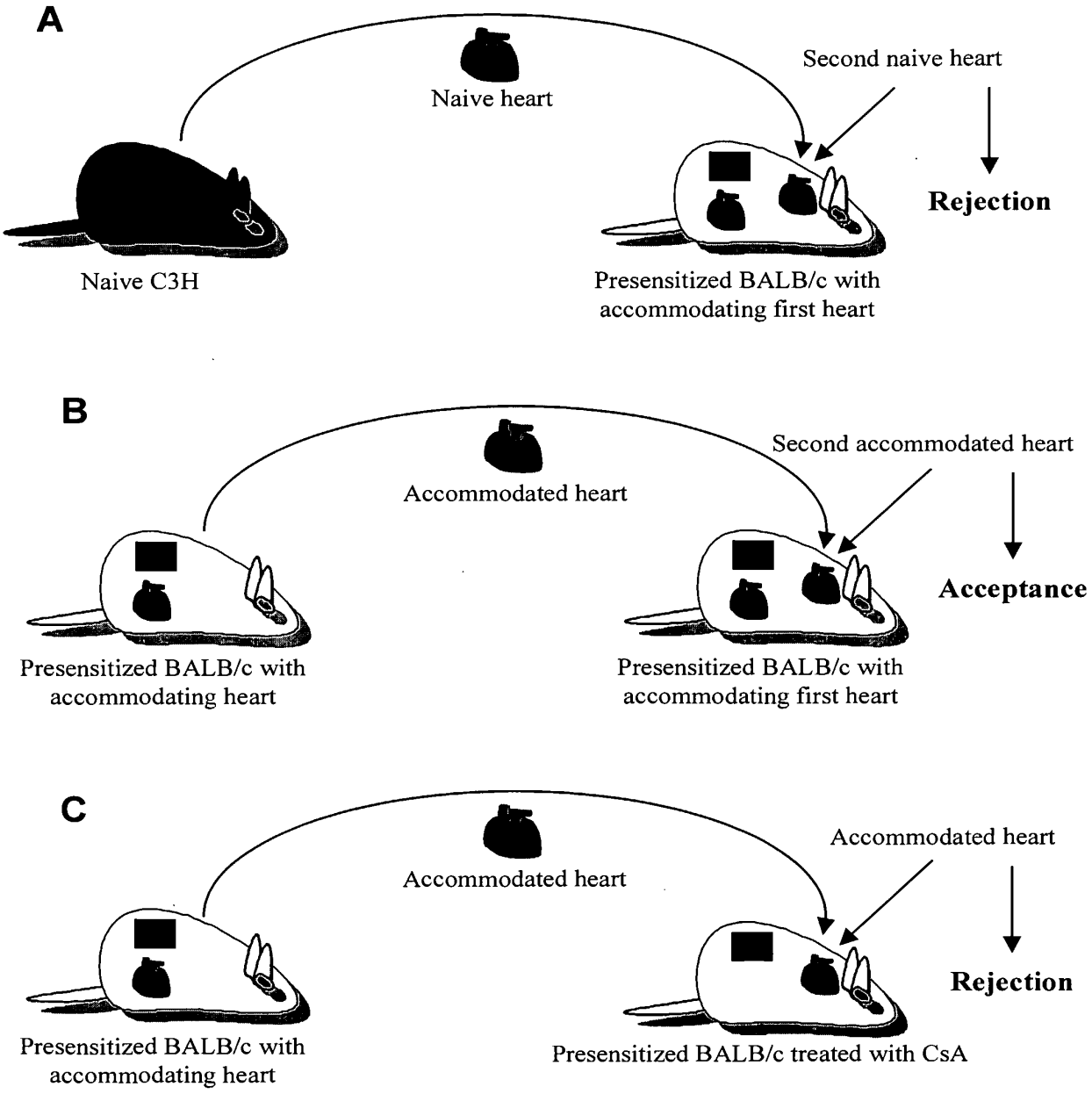
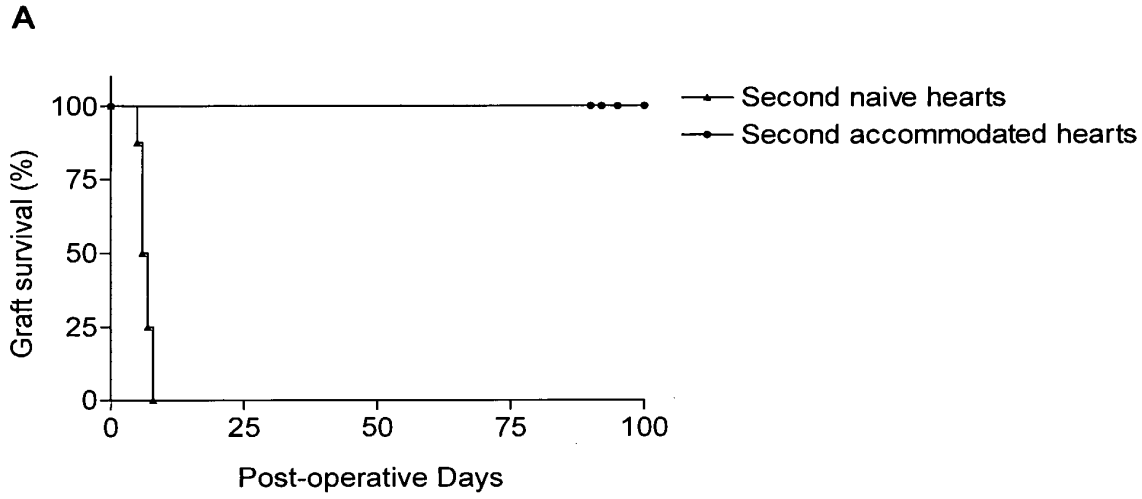


Figure 7

Samsung Bioepis Exhibit No. 1002
Page 1349



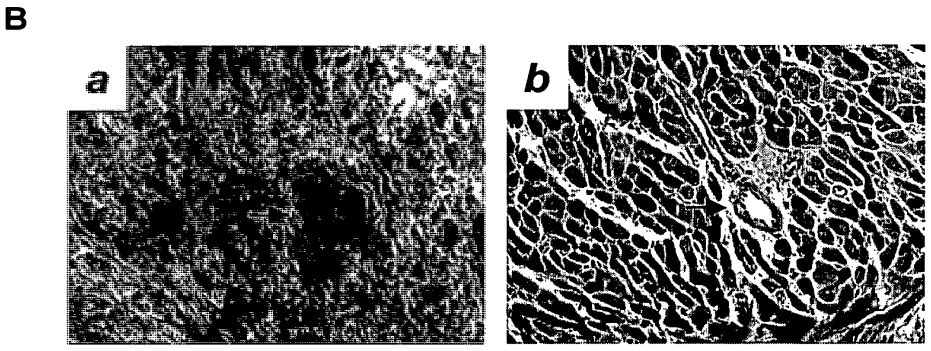


Figure 8

Samsung Bioepis Exhibit No. 1002
Page 1350

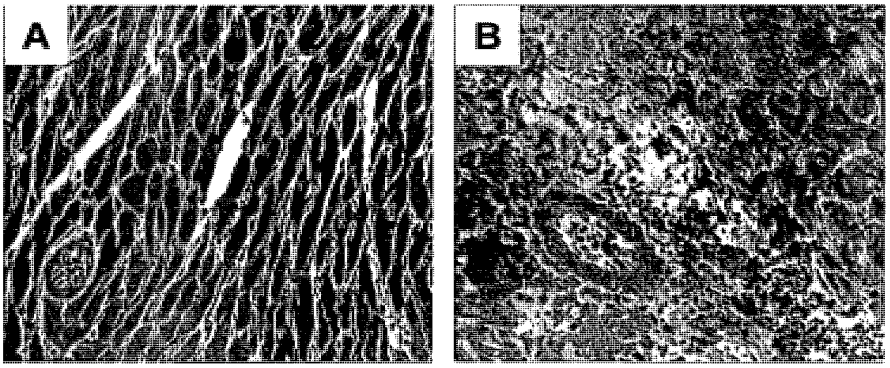


Figure 9

Samsung Bioepis Exhibit No. 1002
Page 1351

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: February 15, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/148,839

Confirmation No.: 3159

Filed: May 6, 2016

Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT (SIDS)

Dear Sir:

In compliance with 37 C.F.R. § 1.56, 1.97 and 1.98, the attention of the Patent and Trademark Office is hereby directed to the documents listed on the attached PTO/SB/08. In accordance with 37 C.F.R. § 1.98(a)(2)(i)-(iv), Applicant submits herewith copies of foreign patents and non-patent literature references, but has not included copies of U.S. patents and U.S. patent applications.

It is respectfully requested that the documents listed on the PTO/SB/08 be expressly considered by the Examiner during the prosecution of this application, and that the documents be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

Applicant calls to the attention of the Examiner the following Applications and provides copies of Office Action cited therein. Applicant also provides a copy of an Office Action from an Application previously made of record:

Applications				
Examiner's Initials	*Serial No.*	*Filing Date*	*First Named Inventor*	*Docket No.*
	15/260,888	09-09-2016	Leonard BELL	AXJ-114BUSCN3
	15/284,015	10-03-2016	Leonard BELL	AXJ-114BUSCN4

Office Actions (copies enclosed)			
Examiner's Initials	Serial No.	Date Mailed from USPTO	Examiner
	15/260,888	12-22-2016	J. Rogers
	15/260,888	10-27-2016	J. Rogers
	15/284,015	11-01-2016	J. Rogers
	13/335,518	08-22-2016	P. Gambel

/JAMES L ROGERS/ 03/21/2017

The Examiner is requested to review the file histories of these applications, including cited references, Office Actions, Responses, etc., and is asked to contact Applicant's Attorney if the Examiner would like the Applicant to supply copies of any or all of the information included in any of these applications. For any of these applications, if Applicant's Attorney is not contacted by the Examiner with such a request, then it will be concluded that the Examiner has reviewed or will review the file content of these applications.

Applicant respectfully requests that the Examiner initial the blank columns next to the cited Applications and Office Actions, to indicate that the information has been considered by the Examiner. Alternatively, Applicant requests that the Examiner insert the phrase, "All references considered except where lined through," on each page of the Information Disclosure Statement, along with the Examiner's initials.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

The filing of this Information Disclosure Statement is not to be interpreted as a representation that the cited documents are material, that an exhaustive search has been conducted, or that no other relevant information exists. Nor shall the citation of any documents herein be construed *per se* as a representation that such document is prior art. Moreover, Applicant understands the Examiner will make an independent evaluation of the cited documents.

This Information Disclosure Statement is filed after the mailing date of a first Office Action on the merits, but before the mailing date of any of a Final Action under 37 C.F.R. § 1.113, a Notice of Allowance under 37 C.F.R. § 1.311 or an action that otherwise closes prosecution in this application (37 C.F.R. § 1.97(c)).

Please charge our Deposit Account No. 12-0080 in the amount of $180.00 covering the fee set forth in 37 C.F.R. § 1.17(p). The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN2.

Dated: February 15, 2017

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 217-4699 (Fax)
Attorney/Agent For Applicant

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

To: chris.schlauch@nelsonmullins.com,ipqualityassuranceboston@nelsonmullins.com,ipboston.docketing@nel
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 133485

Apr 04, 2017 04:17:26 AM

Dear PAIR Customer:

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 133485 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:
The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
15148839	CTNF	04/04/2017	AXJ-114BUSCN2
	892	04/04/2017	AXJ-114BUSCN2
	OA.APPENDIX	04/04/2017	AXJ-114BUSCN2
	OA.APPENDIX	04/04/2017	AXJ-114BUSCN2
	1449	04/04/2017	AXJ-114BUSCN2
	OA.APPENDIX	04/04/2017	AXJ-114BUSCN2
	1449	04/04/2017	AXJ-114BUSCN2

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/148,839	05/06/2016	Leonard BELL	AXJ-114BUSCN2	3159

133485 7590 04/18/2017

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER
ROGERS, JAMES L

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
04/18/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

Applicant-Initiated Interview Summary	**Application No.** 15/148,839	**Applicant(s)** BELL ET AL.	
	Examiner JAMES ROGERS	**Art Unit** 1644	

All participants (applicant, applicant's representative, PTO personnel):

(1) *JAMES ROGERS*.

(2) *DANIEL KOLKER, SPE*.

(3) *PHIL GAMBEL*.

(4) *JANE REMILLARD*

(5) JILL SLOPER

.

Date of Interview: *05 April 2017*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: _____.

Identification of prior art discussed: _____.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

In addition to the above named participants, Todd Spaldin, Darrel Fontenot and Tara Rahemba from Alexion were present for the applicants. The applicant and examiners discussed the Rule 37 CFR 1.105 Request for Additional Information contained in the last office action. The examiners provided suggestions on possible forms of a response..

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/JAMES ROGERS/ Examiner, Art Unit 1644	/DANIEL E. KOLKER/ Supervisory Patent Examiner, Art Unit 1644

Samsung Bioepis Exhibit No. 1002
Page 1358

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

To: chris.schlauch@nelsonmullins.com,ipqualityassuranceboston@nelsonmullins.com,ipboston.docketing@nel
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 133485

Apr 18, 2017 03:42:33 AM

Dear PAIR Customer:

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 133485 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:
The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
15148839	INTV.SUM.APP	04/18/2017	AXJ-114BUSCN2

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/148,839	05/06/2016	Leonard BELL	AXJ-114BUSCN2	3159

133485 7590 05/11/2017

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER
ROGERS, JAMES L

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
05/11/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

Applicant-Initiated Interview Summary	Application No.	Applicant(s)	
	15/148,839	BELL ET AL.	
	Examiner	Art Unit	
	JAMES ROGERS	1644	

All participants (applicant, applicant's representative, PTO personnel):

(1) *JAMES ROGERS*.

(2) *DANIEL KOLKER, SPE*.

(3) *JILL SLOPER*.

(4) *JANE REMILLARD*.

Date of Interview: *01 May 2017*.

Type: ☐ Telephonic ☐ Video Conference
☒ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☒ Yes ☐ No.
If Yes, brief description: *TABLES RE: LISTING OF CLINICAL TRIALS*.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☐Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *1*.

Identification of prior art discussed: _____.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

In addition to the above named participants, Phil Gambel was present for the USPTO and Tera Rahemba, Todd Spalding, and Darrel Fontenot from Alexion were also present. All were present in-person except James Rogers, who joined by video conference.
A follow up to the interview was conducted on 5/5/2017. All of the same participants were present except for Phil Gambel.

The applicant and examiners discussed the Rule 37 CFR 1.105 Request for Additional Information contained in the last office action, and applicant's proposed response. The examiners stated that a rule 132 declaration would be helpful to place all of the documents into context.

The examiners also noted that while no 35 USC 102(a) is on record, the "known or used by others" provisions of 102(a) require public knowledge or use. Thus, if the examiner's determine that there is no 102(b) use after consideration of applicant's proposed response, there will also be no 102(a) use.

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/JAMES ROGERS/ Examiner, Art Unit 1644	/DANIEL E. KOLKER/ Supervisory Patent Examiner, Art Unit 1644

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

To: chris.schlauch@nelsonmullins.com,ipqualityassuranceboston@nelsonmullins.com,ipboston.docketing@nel
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 133485

May 11, 2017 03:42:22 AM

Dear PAIR Customer:

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 133485 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:
The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
15148839	INTV.SUM.APP	05/11/2017	AXJ-114BUSCN2

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: May 12, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/148,839 Confirmation No.: 3159

Filed: May 6, 2016 Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO NON-FINAL OFFICE ACTION UNDER 37 C.F.R. § 1.111

Dear Sir:

In response to the Office Action dated April 4, 2017 (Paper No. 20170227), please amend the above-identified U.S. patent application as follows:

A Listing of the Claims begins on page 2 of this paper.

Remarks/Arguments begin on page 3 of this paper.

LISTING OF THE CLAIMS

1. **(Previously Presented)** A pharmaceutical composition for use in treating a patient afflicted with paroxysmal nocturnal hemoglobinuria (PNH), wherein the composition is a sterile, preservative free, 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml antibody solution, wherein the antibody comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4.

2. **(Previously Presented)** A pharmaceutical composition comprising an anti-C5 antibody, wherein the anti-C5 antibody comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4.

3. **(Previously Presented)** The pharmaceutical composition of claim 2, wherein the pharmaceutical composition is a sterile, preservative free 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution.

Samsung Bioepis Exhibit No. 1002
Page 1366

REMARKS

Claims 1-3 are pending.

Interview Summary

Applicants thank the Examiner and his Supervisor for the interview held on May 1, 2017 and follow-up discussion on May 5, 2017, during which the outstanding rejections and related Rule 37 C.F.R. § 1.105 Request for Additional Information in the above-referenced application were discussed. The substance of the interview and follow-up discussion is reflected in the following remarks.

Moreover, as confirmed in the Interview Summary dated May 11, 2017, if the Examiners determine after consideration of the present response and accompanying Declaration that there was no 35 U.S.C. § 102(b) "public use" of the claimed invention, then there also was no 35 U.S.C. § 102(a) "knowledge or use" of the claimed invention.

Information Disclosure Statement

Applicants acknowledge with appreciation that the Information Disclosure Statements filed on May 6, 2016 and February 15, 2017 have been considered by the Examiner. Additionally, Applicants submit herewith a further Supplemental Information Disclosure Statement to make of record additional information for the Examiner's consideration.

Rejection of Claim 2 Under 35 U.S.C. § 102(b)

The Examiner has rejected claim 2 as allegedly being anticipated by the following references, which the Examiner has characterized as follows:

Cited Reference	Examiner's Characterization
Hillmen *et al.* (*N. Engl. J. Med.* 2004; 350; 552-9), as evidenced by Applicant's specification as filed at page 44 and Thomas *et al.* (*Molecular Immunology*, 33 (17/18), pp. 1389-1401, 1996)	Hillmen *et al.* teaches a method for treating a human patient that suffers from PNH by administering to the patient an effective amount of eculizumab. Applicant's specification at page 44 discloses that SEQ ID NOs: 2 and 4 constitute the heavy and light chains of eculizumab. Thomas *et al.* teach the humanized h5G1.1 antibody, which retains the affinity and blocking functions of its murine counterpart.

Appel *et al*. (*Kidney International*, 70, S45-S50, 2006)	Appel *et al*. teaches that eculizumab is a humanized monoclonal antibody that prevents the activation of complement component C5.
Wang (US2005/0271660)	Wang teaches that eculizumab inhibits activity of the complement system.

The Examiner acknowledges that none of the cited references specifically teaches that eculizumab comprises heavy and light chains consisting of the amino acid sequences set forth SEQ ID NO:2 and SEQ ID NO:4, respectively. However, the Examiner is of the opinion that, absent evidence to the contrary, these heavy and light chain sequences are "inherent properties" of eculizumab.

Applicants respectfully traverse the rejection. As explained in the April 4, 2017 Office Action on pages 3-4, the premise of the Examiner's inherency rejection is that eculizumab (and thus, its full length sequence) "was effectively in the public domain", as evidenced by Exhibit A (studies 41-60 from clinicaltrials.gov), Exhibit B (printout for NCT00005571), Exhibit C (clinicaltrials.gov from 6/23/2005 for NCT00098280), Exhibit D (Kaplan 2002), Exhibit E (Appel *et al.* 2006), Exhibit F (Wang, US20050271660), Exhibit G (Rother, WO 2005/110481), and Exhibit H (Rother, US 2009/0028850). However, this is incorrect. Neither eculizumab nor its complete sequence, including the sequence of its unique, non-naturally occurring, protein-engineered heavy chain, was in the public domain prior to the March 15, 2007 effective filing date of the present application, as discussed in detail below. Accordingly, the presently claimed pharmaceutical composition comprising eculizumab (and the specific sequences thereof) was also not in the public domain prior to that time.

A. Exhibits A-C: Clinical Trials

With respect to the clinical trials cited by the Examiner in Appendices A-C, these clinical trials did not constitute "public use" of eculizumab under pre-AIA 35 U.S.C. § 102(b), as they were controlled, restricted, and subject to strict confidentiality obligations. Specifically, as discussed in the enclosed Declaration Pursuant to 37 C.F.R. § 1.132 by Dr. Laural Boone, the cited clinical trials using eculizumab were strictly controlled and governed by clinical trial protocols which included explicit confidentiality requirements. The Federal Circuit has clearly held that clinical trials performed under such confidentiality and restrictions do not constitute

"public use" within the meaning of 35 U.S.C. § 102(b). In *Dey, L.P. v. Sunovion Pharmaceuticals, Inc.* (No. 2012-1428, Fed. Cir. May 20, 2013) the Federal Circuit considered whether a clinical trial constitutes an invalidating "public use" under 35 U.S.C. § 102(b). Sunovion, the accused infringer, asserted that its own clinical trial was an invalidating public use against Dey's patents, because they involved the accused formulation and took place before Dey's 102(b) critical date. The Federal Circuit concluded that the trial was not an invalidating public use of the study medication based on the following factors: the nature of the activity that occurred in public; the public access to and knowledge of the public use; and whether there was any confidentiality obligation imposed on persons who observed the use (citing *Bernhardt, L.L.C. v. Collezione Europa USA, Inc.*, 386 F.3d 1371, 1379 (Fed. Cir. 2004)). Applying this standard, the Federal Circuit reversed the district court's grant of summary judgment to Sunovion because the clinical trial's restrictions, instructions, and records requirements supported a conclusion ***that the trial was sufficiently controlled and restricted and therefore, not an invalidating public use***. In addition, the Court noted that the ***investigators (i.e., "the most knowledgeable persons involved in the study") were under express confidentiality restrictions, and neither the patients nor the doctors were aware of the claimed particulars of Sunovion's formulation*** (page 10 of *Dey*). Specifically, "while it is true that participants were permitted to discuss the study with their doctors, ***they were not in a position to reveal the composition…because they were unaware of the specifics of the inventive formulations***" (page 10 of *Dey*). As argued by Dey and agreed by the Court, "***if members of the public are not informed of, and cannot readily discern, the claimed features of the invention in the allegedly invalidating prior art, the public has not been put in possession of those features***."

Similar to the facts in *Dey*, the trials cited in Exhibits A-C on page 3 of the Office Action were tightly controlled, restricted and governed by express confidentiality provisions, as discussed in detail in the enclosed Declaration Pursuant to 37 C.F.R. § 1.132 by Dr. Laural Boone. Moreover, similar to the facts in *Dey*, the clinical trial protocols did not contain the specifics of the claimed invention or allow the participants to readily discern the features of the presently claimed invention, including the sequence of the antibody being administered, *i.e.*, eculizumab. Thus, in accordance with *Dey*, the cited clinical trials did not constitute "public use" of eculizumab under pre-AIA 35 U.S.C. § 102(b).

B. Exhibits D-H: Publications

With respect to the publications cited in Exhibit D (Kaplan 2002), Exhibit E (Appel *et al.* 2006), Exhibit F (Wang, US20050271660), Exhibit G (Rother, WO 2005/110481), and Exhibit H (Rother, US 2009/0028850), the Examiner is of the opinion that these publications demonstrate that "eculizumab was effectively in the public domain" prior to the effective filing date of the present application. In support of this assertion, the Examiner cites *In re Antor Media Corp* (CAFC, 2012) for the proposition that "the subject matter disclosed in a reference, whether it is a ***patent claim***, ***other parts of a patent***, or non-patent literature, is presumed to be enabling absent evidence to the contrary (emphasis added)."

Applicants respectfully disagree with the Examiner's position. From the outset, as noted by the Court in *In re Antor Media Corp*, the question of enablement only arises if the cited reference in fact teaches each and every element of the claimed invention (page 9 of *In re Antor Media Corp*). In the present case, none of the publications cited in Exhibits D-H teach the complete structure of eculizumab, as presently claimed, including its complete sequence with a unique, non-naturally occurring, protein-engineered heavy chain. Nor do these references enable the presently claimed full biochemical structure of eculizumab, for the reasons discussed above. Exhibit D (Kaplan 2002) describes clinical studies that used eculizumab. Exhibit E (Appel *et al.* 2006) merely discloses that eculizumab is a humanized monoclonal antibody that prevents the activation of complement component C5. Exhibit F (Wang, US20050271660) discloses antibody formulations suitable for nebulization using murine antibodies in the examples. Exhibit G (Rother, WO 2005/110481) and Exhibit H (Rother, US 2009/0028850, which was not published until after March 16, 2007) disclose the use of eculizumab for treating PNH patients, as well as the use of the mouse anti-C5 antibody BB5.1 in prolonging the survival of an allograft. Importantly, none of the cited references teach the sequence of eculizumab, let alone its unique, non-naturally occurring, protein-engineered full heavy chain (including the CH1-hinge-CH2-CH3 regions).

As discussed above, to anticipate a claimed invention, a reference must provide an enabling disclosure of the desired subject matter. Specifically, pursuant to M.P.E.P § 2121.1, "***mere naming or description of the subject matter is insufficient,*** if it cannot be produced without undue experimentation. *Elan Pharm., Inc. v. Mayo Found. For Med. Educ. & Research,*

346 F.3d 1051, 1054, 68 USPQ2d 1373, 1376 (Fed. Cir. 2003) (emphasis added)." Further, a "reference contains an 'enabling disclosure' if the public was in possession of the claimed invention before the date of invention." Such "***possession is effected if one of ordinary skill in the art could have combined the publication's description of the invention with his [or her] own knowledge to make the claimed invention***. *In re Donohue,* 766 F.2d 531, 226 USPQ 619 (Fed. Cir. 1985) (emphasis added)."

It is clear that the public was not in possession of the claimed sequences of eculizumab prior to the effective filing date of the present application by virtue of the cited publications, because the ***mere naming of eculizumab is insufficient to enable*** the claimed sequences and ***one of ordinary skill in the art could not have readily determined the sequences based on the limited disclosure in the publications and his or her own general knowledge***.

C. Conclusion

In sum, none of the cited references teach or enable the complete sequence of eculizumab, as presently claimed. Moreover, eculizumab was not in "public use" under 35 U.S.C. § 102(b) prior to the March 15, 2007 effective filing date of the present application, at least because the clinical trials cited by the Examiner were tightly controlled, restricted, governed by confidentiality obligations, and conducted according to protocols that did not disclose the claimed features of the invention (see *Dey, L.P. v. Sunovion Pharmaceuticals, Inc.*). Accordingly, it is clear that the public was not in possession of the claimed sequences of eculizumab prior to the effective filing date of the present application, and the Examiner is respectfully requested to reconsider and withdraw the rejection.

Rejection of Claims 1-3 Under 35 U.S.C. § 103(a)

Claims 1-3 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Hillmen *et al.* (*N. Engl. J. Med.* 2004; 350; 552-9), in view of Evans *et al.* (US 6,355,245) and Wang (2005/0271660), and as evidenced by the parent specification (US Patent Application No.: 13/426,973). The Examiner asserts that Applicants' argument that the complete structure of eculizumab was not disclosed in the art or available to the public is unpersuasive because "the preponderance of evidence indicates that the antibody was in public use" and this public

availability was "sufficient to have placed one of skill in the art in possession of applicant's currently claimed sequences of eculizumab."

Applicants respectfully traverse the rejection. As discussed above, neither eculizumab nor its complete sequence were available to the public or in "public use" prior to the March 15, 2007 effective filing date of the present application. Therefore, the Examiner's underlying premise supporting the present obviousness rejection (*i.e.*, that eculizumab was in "public use") is factually incorrect. Moreover, as acknowledged by the Examiner on page 7 of the Office Action, none of Hillmen *et al.*, Evans *et al.* or Wang teach or suggest the complete sequence of eculizumab. Clearly, the Examiner has failed even to establish a prima facie case of obviousness.

Accordingly, for at the least the above-discussed reasons, the pending claims are patentable and the Examiner is respectfully requested to reconsider and withdraw this rejection.

Obviousness-Type Double Patenting

The Examiner has rejected claims 1-3 on the ground of non-statutory obviousness-type double patenting as being unpatentable over:

1. Claims 1-9 of US Patent No: 9,494,601 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
2. Claims 1-18 of US Patent No: 8,999,340 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
3. Claims 1-17 of US Patent No: 9,409,980 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
4. Claims 3-14 of US Patent No: 9,556,263 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
5. Claims 1-22 of US Patent No: 9,352,035 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
6. Claims 1-14 of US Patent No: 9,415,102 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
7. Claims 1-17 of US Patent No: 9,409,980 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
8. Claims 1-4 of US Patent No: 9,447,176 in view of Hillmen *et al.*, Evans *et al.*, and Wang;

Samsung Bioepis Exhibit No. 1002
Page 1372

9. Claims 1-5, 7, 9-16 of US Patent No: 9,388,235 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
10. Claims 15-17 and 58-59 of US Patent Application No: 11/127,438 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
11. Claims 1-4 of US Patent Application No: 15/243,386 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
12. Claims 1-4 of US Patent Application No: 15/243,290 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
13. Claims 94 and 97-98 of US Patent Application No: 15/282,464 in view of Hillmen *et al.*, Evans *et al.*, and Wang; and
14. Claims 129-138 of US Patent Application No: 15/148,491 in view of Hillmen *et al.*, Evans *et al.*, and Wang.

While in no way acquiescing to the rejection, Applicants respectfully submit herewith a terminal disclaimer over US Patent No: 9,494,601, US Patent No: 8,999,340**,** US Patent No: 9,409,980,US Patent No: 9,556,263, US Patent No: 9,352,035, US Patent No: 9,415,102, US Patent No: 9,447,176, US Patent No: 9,388,235, US Patent Application No: 15/243,290, US Patent Application No: 15/282,464, and US Patent Application No: 15/148,491.

With respect to US Patent Application No: 15/243,386, Applicants note that this application lapsed on April 20, 2017 for failure to respond to the Office Action dated October 19, 2016. Accordingly, the rejection relating to the '386 application has been rendered moot.

Finally, with respect to US Patent Application No: 11/127,438, while the Examiner acknowledges that "the conflicting claims are not identical", the Examiner asserts that "they are not patentably distinct from each other because both the current application and the '438 claim [application] claim eculizumab." Applicants respectfully traverse this rejection.

The pending claims are drawn to pharmaceutical compositions comprising *eculizumab as defined by the complete heavy and light chain sequences of the antibody*. In contrast, claims 15-17 and 58-59 of the '438 application are drawn to an *aerosol composition* comprising h5G1.1, which is useful for the treatment of asthma. Importantly, the claims of the '438 application fail to recite any part of the sequence of eculizumab, let alone the complete heavy and light chain sequences. Nor does the specification of the '438 application teach, enable or in any way render

Samsung Bioepis Exhibit No. 1002
Page 1373

obvious the complete structure of eculizumab, as presently claimed. Moreover, as discussed above and in the attached Declaration, neither eculizumab nor its complete sequence were available to the public or in "public use" prior to the March 15, 2007 effective filing date of the present application. Accordingly, the mere naming of h5G1.1 in the claims of the '438 application does not render the present claims anticipated by or obvious over those of the '438 application.

A "nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s)" (see MPEP § 804). In view of the above, it is clear that the pending claims of the present '839 application and the pending claims of the '438 application are ***patentably distinct***. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

Response to Request for Additional Information Under 37 C.F.R. § 1.105

In response to the Examiner's specific inquiries and request for additional information under 37 C.F.R. § 1.105, Applicants provide the following factual information, as well as the enclosed Declaration Pursuant to 37 C.F.R. § 1.132 by Dr. Laural Boone.

(1) Interrogatories A–F

As attested in the enclosed Declaration, Exhibits A through F cited by the Examiner on pages 7-9 of the Office Action reference 17 different clinical studies using eculizumab that occurred prior to March 16, 2007. Of the 17 studies, 4 are referred to as "extension" studies, which are studies that are appended to a clinical study to prolong the treatment and/or monitoring period of the same patients that participated in the initial study.

Of these 17 clinical studies, one that is described in both Exhibits D and F and entitled, "A Randomized, Third-Party-Blind, Placebo-Controlled, Pilot Study of the Effect of h5G1.1-mAb in Patients with Bullous Pemphigoid," only enrolled one patient, and that one patient was randomized to receive placebo. Thus, no patient received eculizumab in the course of this study and the study was terminated.

For the remaining 16 clinical studies identified in Exhibits A through F, the below table includes: the full name of each study (and extension study thereof, if applicable, identified by an "E" or an "X" in the study number), the study number(s), the Exhibit(s) cited by the Examiner on pages 7-8 of the Office Action that each study was described in, and whether the study (including any extension study thereof) included a confidentiality provision (as described in further detail below).

Study Name[1]	**Study Number**	**Exhibit(s)**	**Explicit Confidentiality Provision**
An Open-Label Study of the Effect of Eculizumab (h5G1.1-mAb) on Patients with Transfusion Dependent, Hemolytic, Paroxysmal Nocturnal Hemoglobinuria	C02-001	F	Yes
Extension Study of Eculizumab in Patients with Transfusion Dependent, Haemolytic, Paroxysmal Nocturnal Haemoglobinuria Who Previously Participated in Study C02-001	E02-001		
An Open-Label Extension Study of Eculizumab in Patients with Transfusion Dependent, Haemolytic, Paroxysmal Nocturnal Hemoglobinuria Who Previously Participated in Study E02-001	X03-001		
A Hemoglobin Stabilization and Transfusion Reduction Efficacy and Safety Clinical Investigation, Randomized, Multi-Center, Double-Blind, Placebo-Controlled, Using Eculizumab in Paroxysmal Nocturnal Hemoglobinuria Patients (TRIUMPH)	C04-001	A[2], F	Yes
Safety in Hemolytic PNH Patients Treated with Eculizumab: A Multi-Center Open-Label Research Design Study (SHEPHERD)	C04-002	A, C, F	Yes

[1] Although some of these studies may not have occurred in the United States and thus are not available to give rise to public use under 35 U.S.C. § 102(b), all of the trials have been in included in this table (regardless of where they occurred).

[2] Of the studies referenced in Exhibit A, studies 41-43, 45-46, 52-54, 56-58 and 60 are all indicated as having been "received" or "started" *after* March 16, 2007, *i.e.*, the date eculizumab was first approved for commercial marketing and sale. Thus, only studies identified as numbers 44, 47-51, 55 and 59 in Exhibit A are included in this table.

Samsung Bioepis Exhibit No. 1002
Page 1375

Study Name[1]	Study Number	Exhibit(s)	Explicit Confidentiality Provision
A Phase III, Open-Label, Extension Study of Eculizumab in Patients with Transfusion Dependent, Hemolytic Paroxysmal Nocturnal Hemoglobinuria (PNH) Who Have Participated in the TRIUMPH (C04-001), SHEPHERD (C04-002) or X03-001 Studies	E05-001	A, F	Yes
A Randomized, Double-Blind, Placebo-Controlled Pilot Study of the Effect of h5G1.1-mAb on Dermatomyositis Patients	C99-006	B, D[3], F	Yes
Phase I Study of h5G1.1-mAb in Patients with Rheumatoid Arthritis	C97-001	F	Yes
A Phase II Randomized, Double-Blind, Placebo-Controlled Study of the Effect of h5G1.1-mAb on Rheumatoid Arthritis Patients Receiving Methotrexate	C99-001	D, F	Yes
A Phase IIB Randomized, Double-Blind, Placebo-Controlled Study of the Effect of h5G1.1-mAb on Rheumatoid Arthritis (RA) Patients with Active Disease Despite Receiving Methotrexate or Leflunomide	C01-004	D, F	Yes
A Phase II Randomized, Double-Blind, Placebo-Controlled Study of the Effect of h5G1.1-mAb on the Reduction of Proteinuria in Patients with Idiopathic Membranous Glomerulopathy An Open Label Study of the Long-Term Safety of h5G1.1-mAb on the Reduction of Proteinuria in Patients with Idiopathic Membranous Glomerulopathy	C99-004 E99-004	D, E, F	Yes
Phase I Study of h5G1.1-mAb in Patients with Systemic Lupus Erythematosus	C97-002	D, F	Yes
A Randomized, Double-Blind, Placebo-Controlled Pilot Study of the Effect of h5G1.1-mAb on Psoriatic Patients	C99-007	D, F	Yes
The Paroxysmal Nocturnal Hemoglobinuria Early Access Treatment Protocol (EMBRACE)	C06-002	F	Yes
PNH Registry	M07-001	A, F	Yes

[3] Item 445382 referenced in the Examiner's question regarding the Kaplan reference (Exhibit D) does not refer to an actual study. However, to address any potential questions the Examiner has with respect to Exhibit D, the Kaplan reference has been reviewed in its entirety and the clinical studies described therein that used eculizumab have been included.

(i) The Antibody Sequences Used in the Clinical Studies

As attested in the enclosed Declaration, the antibody (eculizumab) used in each of the studies included in the above table contained the heavy and light chain sequences of SEQ ID NOs: 2 and 4. However, the protocols used to conduct those studies did not inform either doctors or patients of the sequences.

(ii, iii) Confidentiality Provisions in the Clinical Studies

As indicated in the table above and discussed in the enclosed Declaration, in the course of conducting each of the clinical trials listed, participating physicians and others involved in the study were required to maintain Alexion's confidential and/or proprietary information in strict confidence. For example, these individuals were required to maintain the confidentiality of the clinical trial protocols themselves, as each protocol was marked "Confidential". These individuals were also bound by explicit confidentiality provisions in the protocol. As attested to in the attached Declaration of Dr. Boone, the following language from the protocol for study number C04-001, which refers to the Phase III TRIUMPH study, provides a representative example of the confidentiality requirements set forth directly within these clinical study protocols:

> "THE INVESTIGATOR AND ALL EMPLOYEES AND COWORKERS INVOLVED WITH THIS STUDY SHALL NOT DISCLOSE OR USE FOR ANY PURPOSE, OTHER THAN PERFORMANCE OF THE STUDY, ANY DATA, RECORDS OR OTHER UNPUBLISHED, CONFIDENTIAL INFORMATION DISCLOSED TO THOSE INDIVIDUALS FOR THE PURPOSE OF THE STUDY. PRIOR WRITTEN AGREEMENT FROM THE SPONSOR OR ITS DESIGNEE MUST BE OBTAINED FOR THE DISCLOSURE OF ANY SAID CONFIDENTIAL INFORMATION TO OTHER PARTIES."

As another representative example, Dr. Boone cites the protocol for the study in dermatomyositis (study number C02-002), which stated:

> "THIS CONFIDENTIAL INFORMATION ABOUT AN INVESTIGATIONAL DRUG IS PROVIDED FOR THE EXCLUSIVE USE OF INVESTIGATORS

> OF THIS DRUG AND IS SUBJECT TO RECALL AT ANY TIME. THE INFORMATION IN THIS DOCUMENT MAY NOT BE DISCLOSED UNLESS SUCH DISCLOSURE IS REQUIRED BY FEDERAL OR STATE LAW OR REGULATIONS SUBJECT TO THE FOREGOING. THIS INFORMATION MAY BE DISCLOSED ONLY TO THOSE PERSONS INVOLVED IN THE STUDY WHO HAVE A NEED TO KNOW WITH THE OBLIGATION NOT TO FURTHER DISSEMINATE THIS INFORMATION. THESE RESTRICTIONS ON DISCLOSURE WILL APPLY EQUALLY TO ALL FUTURE ORAL OR WRITTEN INFORMATION SUPPLIED TO YOU BY ALEXION, WHICH IS DESIGNATED AS 'PRIVILEGED' OR 'CONFIDENTIAL'."

As Dr. Boone explains, all confidentiality provisions in the protocols for the studies listed in the table above were substantively identical to these representative confidentiality provisions from studies C04-001 and C02-002.

In addition, Dr. Boone attests that each clinical trial protocol required strict control over the physical drug product (eculizumab). For example, the clinical trial protocol for the TRIUMPH study described the secure storage and inventory and disposal requirements of each participating physician and study site as shown below:

> "Eculizumab must be stored in a secure, limited-access storage area. . . .
>
> Test Article Accountability…When a drug shipment is received at the site, the pharmacist at each site shall verify the contents, sign the packing invoice provided with the shipment, and maintain the original copy for review by the study monitor. The duplicate copy should be kept in the pharmacy binder.
>
> Each shipment of investigational drug supplies will contain an accountability log to assist the pharmacist in maintaining current and accurate inventory records covering receipt, dispensing, and disposition of the investigational product. During the study, the following information must be noted in the accountability log: the identification number(s), initials of patient(s) who is dispensed investigational product, the date(s) that the investigational product is prepared and dispensed, and the initials of the pharmacist or designee who prepared the investigational product. Empty vials and vials with residual materials should be kept for inspection by the study monitor.
>
> The study monitor will examine the inventory records during study conduct. Additionally, the inventory records must be readily available and may be subject

> to regulatory authorities or an independent auditor's inspection at any time. At the completion of the study, to satisfy regulatory requirements regarding drug accountability, all remaining investigational product inventory will be reconciled and retained or destroyed according to applicable local, state, and federal regulations."

Dr. Boone also explains that all of the protocols for the studies listed in the above table included similar (if not identical) drug storage and accountability provisions, except for the PNH Registry protocol (the last entry in the table above). The PNH Registry was not a study in which eculizumab was administered to any patients; rather, it was a "non-interventional study designed to collect post-marketing pharmacovigilance and effectiveness data on patients treated with Soliris® (eculizumab)" and to "collect information on the progression of disease in the Soliris treated and untreated PNH patient population." Accordingly, the protocol for the PNH Registry did not contain drug storage or accountability provisions. The PNH Registry protocol did, however, include strict confidentiality provisions as indicated in the table above, *e.g.,* requiring all physicians to "agree to maintain the confidentiality of all information received or developed in connection with [the] protocol."

(iv) Physicians Acting at Alexion's Direction

As discussed in the enclosed Declaration, to participate in each clinical trial listed in the table above, each physician had to sign the protocol for the trial, committing to adhere to the respective clinical trial protocol's provisions. For example, the TRIUMPH study protocol, just prior to the physician's signature line, stated:

> "I agree to conduct the study as outlined in the protocol entitled: *"TRIUMPH*: A Hemoglobin Stabilization and Transfusion Reduction Efficacy and Safety Clinical Investigation, Randomized, Multi-Center, Double-Blind, Placebo-Controlled, Using Eculizumab in Paroxysmal Nocturnal Hemoglobinuria Patients" and in accordance with the guidelines and all applicable government regulations. I have read and understand all sections of the protocol including Section 11, Administrative Considerations."

As Dr. Boone states, all of the other protocols similarly required each physician (or investigator) to agree to conduct the study in accordance with the protocol.

(2) Interrogatory G

As attested in the enclosed Declaration, the antibody having the heavy chain described by SEQ ID NO:2 and the light chain described by SEQ ID NO:4 was first approved for commercial marketing and sale on March 16, 2007.

CONCLUSION

In view of the above amendment, arguments, and accompanying Declaration, Applicants believe the pending application is in condition for allowance. If a telephone conversation with Applicants' attorney would help expedite the prosecution of the above-identified application, the Examiner is urged to call Applicants' attorney at (617) 217-4630.

Applicants believe no fee is required with this submission. However, should any additional fee be due, the Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN2.

Dated: May 12, 2017

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 217-4699 (Fax)
Attorney/Agent For Applicant

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: May 12, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/148,839

Confirmation No.: 3159

Filed: May 6, 2016

Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION PURSUANT TO 37 C.F.R. § 1.132 BY DR. LAURAL BOONE

I, Laural S. Boone, J.D., Ph.D., declare the following:

1. I am presently a Senior Director and Patent Counsel at Alexion Pharmaceuticals, Inc., (hereafter, "Alexion") which is located in New Haven, Connecticut. I have been employed in this capacity at Alexion for three years to date. Alexion is the Assignee of the above-referenced patent application. I received a B.A. in Chemistry from Barnard College, a M.S. and Ph.D. in Organic Chemistry from the University of Chicago, and a J.D. from the George Washington University Law School. I also completed a Postdoctoral Fellowship in Cellular and Molecular Biophysics at Rockefeller University. I am registered to practice as a patent attorney before the United States Patent & Trademark Office.

Samsung Bioepis Exhibit No. 1002
Page 1381

2. I have reviewed the Examiner's request for additional information under 37 C.F.R. § 1.105 and provide the following statements.

3. I have reviewed Exhibits A through F cited by the Examiner on pages 7-8 of the April 4, 2017 Office Action, including each of the 37 documents listed in Exhibit F. These exhibits and the documents listed therein reference 17 different clinical studies using eculizumab that occurred prior to March 16, 2007. Of the 17 studies, 4 are referred to as "extension" studies, which are studies that are appended to a clinical study to prolong the treatment and/or monitoring period of the same patients that participated in the initial study.

4. Of these 17 clinical studies, one that is described in both Exhibits D and F, is entitled "A Randomized, Third-Party-Blind, Placebo-Controlled, Pilot Study of the Effect of h5G1.1-mAb in Patients with Bullous Pemphigoid." Based on my review of communications between Alexion employees and physicians participating in this study, I have determined that only one patient was enrolled in this study, and that the one patient was randomized to receive placebo. Thus, no patient received eculizumab in the course of this study, and the study was terminated.

5. For the remaining 16 clinical studies identified in Exhibits A through F, I have reviewed each of the clinical trial protocols and the following information from the protocols is shown in the table below: the full name of each study (and extension study thereof, if applicable, identified by an "E" or an "X" in the study number), the study number(s), the Exhibit(s) cited by the Examiner on pages 7-8 of the Office Action that each study was described in, and whether the study (including any extension study thereof) included a confidentiality provision (as described in further detail below).

Samsung Bioepis Exhibit No. 1002
Page 1382

Study Name[1]	Study Number	Exhibit(s)	Explicit Confidentiality Provision
An Open-Label Study of the Effect of Eculizumab (h5G1.1-mAb) on Patients with Transfusion Dependent, Hemolytic, Paroxysmal Nocturnal Hemoglobinuria Extension Study of Eculizumab in Patients with Transfusion Dependent, Haemolytic, Paroxysmal Nocturnal Haemoglobinuria Who Previously Participated in Study C02-001 An Open-Label Extension Study of Eculizumab in Patients with Transfusion Dependent, Haemolytic, Paroxysmal Nocturnal Hemoglobinuria Who Previously Participated in Study E02-001	C02-001 E02-001 X03-001	F	Yes
A Hemoglobin Stabilization and Transfusion Reduction Efficacy and Safety Clinical Investigation, Randomized, Multi-Center, Double-Blind, Placebo-Controlled, Using Eculizumab in Paroxysmal Nocturnal Hemoglobinuria Patients (TRIUMPH)	C04-001	A[2], F	Yes
Safety in Hemolytic PNH Patients Treated with Eculizumab: A Multi-Center Open-Label Research Design Study (SHEPHERD)	C04-002	A, C, F	Yes
A Phase III, Open-Label, Extension Study of Eculizumab in Patients with Transfusion Dependent, Hemolytic Paroxysmal Nocturnal Hemoglobinuria (PNH) Who Have Participated in the TRIUMPH (C04-001), SHEPHERD (C04-002) or X03-001 Studies	E05-001	A, F	Yes
A Randomized, Double-Blind, Placebo-Controlled Pilot Study of the Effect of h5G1.1-mAb on Dermatomyositis Patients	C99-006	B, D[3], F	Yes

[1] Although some of these studies may not have occurred in the United States and thus are not available to give rise to public use under 35 U.S.C. § 102(b), all of the trials are included in this table (regardless of where they occurred).

[2] Of the studies referenced in Exhibit A, studies 41-43, 45-46, 52-54, 56-58 and 60 are all indicated as having been "received" or "started" *after* March 16, 2007, *i.e.*, the date eculizumab was first approved for commercial marketing and sale. Thus, only studies started before the marketing approval, identified as numbers 44, 47-51, 55, and 59 in Exhibit A, are included in this table.

[3] Item 445382 referenced in the Examiner's question regarding the Kaplan reference (Exhibit D) does not refer to an actual study. However, to address any potential questions the Examiner has with respect to

Samsung Bioepis Exhibit No. 1002
Page 1383

Study Name[1]	Study Number	Exhibit(s)	Explicit Confidentiality Provision
Phase I Study of h5G1.1-mAb in Patients with Rheumatoid Arthritis	C97-001	F	Yes
A Phase II Randomized, Double-Blind, Placebo-Controlled Study of the Effect of h5G1.1-mAb on Rheumatoid Arthritis Patients Receiving Methotrexate	C99-001	D, F	Yes
A Phase IIB Randomized, Double-Blind, Placebo-Controlled Study of the Effect of h5G1.1-mAb on Rheumatoid Arthritis (RA) Patients with Active Disease Despite Receiving Methotrexate or Leflunomide	C01-004	D, F	Yes
A Phase II Randomized, Double-Blind, Placebo-Controlled Study of the Effect of h5G1.1-mAb on the Reduction of Proteinuria in Patients with Idiopathic Membranous Glomerulopathy An Open Label Study of the Long-Term Safety of h5G1.1-mAb on the Reduction of Proteinuria in Patients with Idiopathic Membranous Glomerulopathy	C99-004 E99-004	D, E, F	Yes
Phase I Study of h5G1.1-mAb in Patients with Systemic Lupus Erythematosus	C97-002	D, F	Yes
A Randomized, Double-Blind, Placebo-Controlled Pilot Study of the Effect of h5G1.1-mAb on Psoriatic Patients	C99-007	D, F	Yes
The Paroxysmal Nocturnal Hemoglobinuria Early Access Treatment Protocol (EMBRACE)	C06-002	F	Yes
PNH Registry	M07-001	A, F	Yes

Exhibit D, I reviewed the Kaplan reference in its entirety, and the clinical studies described therein that used eculizumab are also included in this table.

Samsung Bioepis Exhibit No. 1002
Page 1384

6. I have reviewed an alignment of the sequence of eculizumab that is listed in the Biologic License Application for Soliris®(eculizumab) ("BLA") with the heavy and light chain sequences of SEQ ID NOs: 2 and 4 of the parent of the pending application, PCT/US2007/006606 (published as WO 2007/106585). I have concluded that the antibody (eculizumab) used in each of the studies included in the above table contained the heavy and light chain sequences of SEQ ID NOs: 2 and 4. However, the protocols used to conduct those studies did not inform either doctors or patients of the sequences.

7. As indicated in the table above, in the course of conducting each of the clinical trials listed, participating physicians and other participants in the study were required to maintain Alexion's confidential and/or proprietary information in strict confidence. For example, these individuals were required to maintain the confidentiality of the clinical trial protocols themselves, as each protocol was marked "Confidential." These individuals were also bound by explicit confidentiality provisions in the protocol. The following language from the protocol for study number C04-001, which refers to the Phase III TRIUMPH study, provides a representative example of the confidentiality requirements set forth directly within these clinical study protocols (emphasis added):

> "THE INVESTIGATOR AND ALL EMPLOYEES AND COWORKERS INVOLVED WITH THIS STUDY SHALL NOT DISCLOSE OR USE FOR ANY PURPOSE, OTHER THAN PERFORMANCE OF THE STUDY, ANY DATA, RECORDS OR OTHER UNPUBLISHED, CONFIDENTIAL INFORMATION DISCLOSED TO THOSE INDIVIDUALS FOR THE PURPOSE OF THE STUDY. PRIOR WRITTEN AGREEMENT FROM THE SPONSOR OR ITS DESIGNEE MUST BE OBTAINED FOR THE DISCLOSURE OF ANY SAID CONFIDENTIAL INFORMATION TO OTHER PARTIES."

8. As another representative example, the protocol for the study in dermatomyositis (study number C02-002) stated:

> "THIS CONFIDENTIAL INFORMATION ABOUT AN INVESTIGATIONAL DRUG IS PROVIDED FOR THE EXCLUSIVE USE OF INVESTIGATORS OF THIS DRUG AND IS SUBJECT TO RECALL AT ANY TIME. THE INFORMATION IN THIS DOCUMENT MAY NOT BE DISCLOSED UNLESS SUCH DISCLOSURE IS REQUIRED BY FEDERAL OR STATE LAW OR REGULATIONS SUBJECT TO THE FOREGOING. THIS INFORMATION MAY BE DISCLOSED ONLY TO THOSE PERSONS INVOLVED IN THE STUDY WHO HAVE A NEED TO KNOW WITH THE OBLIGATION NOT TO FURTHER DISSEMINATE THIS INFORMATION. THESE RESTRICTIONS ON DISCLOSURE WILL APPLY EQUALLY TO ALL FUTURE ORAL OR WRITTEN INFORMATION SUPPLIED TO YOU BY ALEXION, WHICH IS DESIGNATED AS 'PRIVILEGED' OR 'CONFIDENTIAL'."

9. All confidentiality provisions in the protocols for the studies listed in the table above were substantively identical to these representative confidentiality provisions from studies C04-001 and C02-002.

10. In addition, each clinical trial protocol that I reviewed also required strict control over the physical drug product (eculizumab). For example, the clinical trial protocol for the TRIUMPH study described the secure storage and inventory and disposal requirements of each participating physician and study site as shown below:

> "Eculizumab must be stored in a secure, limited-access storage area. . . .
>
> Test Article Accountability…When a drug shipment is received at the site, the pharmacist at each site shall verify the contents, sign the packing invoice provided with the shipment, and maintain the original copy for review by the study monitor. The duplicate copy should be kept in the pharmacy binder.
>
> Each shipment of investigational drug supplies will contain an accountability log to assist the pharmacist in maintaining current and accurate inventory records covering receipt, dispensing, and disposition of the investigational product. During the study, the following information must be noted in the accountability log: the identification number(s), initials of patient(s) who is dispensed investigational product, the date(s) that the investigational product is prepared and dispensed, and the initials of the pharmacist or designee who prepared the investigational product. Empty vials and vials with residual materials should be kept for inspection by the study monitor.

> The study monitor will examine the inventory records during study conduct. Additionally, the inventory records must be readily available and may be subject to regulatory authorities or an independent auditor's inspection at any time. At the completion of the study, to satisfy regulatory requirements regarding drug accountability, all remaining investigational product inventory will be reconciled and retained or destroyed according to applicable local, state, and federal regulations."

11. All of the protocols that I reviewed for the studies listed in the above table included similar (if not identical) drug storage and accountability provisions, except for the PNH Registry protocol (the last entry in the table above). The PNH Registry was not a study in which eculizumab was administered to any patients, but was a registry for all participating patients with PNH either treated or untreated. More particularly, it was a "non-interventional study designed to collect post-marketing pharmacovigilance and effectiveness data on patients treated with Soliris® (eculizumab)" and to "collect information on the progression of disease in the Soliris treated and untreated PNH patient population." Accordingly, the protocol for the PNH Registry did not contain drug storage or accountability provisions. The PNH Registry protocol did, however, include strict confidentiality provisions as indicated in the table above, *e.g.*, requiring all physicians to "agree to maintain the confidentiality of all information received or developed in connection with [the] protocol."

12. To participate in each clinical trial listed in the table above, each physician had to sign the protocol for the trial, committing to adhere to the respective clinical trial protocol's provisions. For example, the TRIUMPH study protocol, just prior to the physician's signature line, states:

> "I agree to conduct the study as outlined in the protocol entitled: *"TRIUMPH*: A Hemoglobin Stabilization and Transfusion Reduction Efficacy and Safety Clinical Investigation, Randomized, Multi-Center, Double-Blind, Placebo-Controlled, Using Eculizumab in Paroxysmal Nocturnal Hemoglobinuria Patients" and in accordance with the guidelines and all applicable government regulations. I have read and understand all sections of the protocol including Section 11, Administrative Considerations."

13. All of the other protocols that I reviewed similarly required each physician (or investigator) to agree to conduct the study in accordance with the protocol.

14. In response to the Examiner's Request (G), based on the BLA approval letter that I reviewed from the Food and Drug Administration Department of Health & Human Services to Alexion regarding eculizumab, the antibody having the heavy chain described by SEQ ID NO: 2 and the light chain described by SEQ ID NO: 4 was first approved for commercial marketing and sale on March 16, 2007.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or of any patent issued thereon.

Laural S. Boone, J.D., Ph.D. 11 MAY 2017
Date

Electronic Patent Application Fee Transmittal

Application Number:	15148839
Filing Date:	06-May-2016
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Attorney Docket Number:	AXJ-114BUSCN2

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
STATUTORY OR TERMINAL DISCLAIMER	1814	2	160	320
		Total in USD ($)		**500**

Electronic Acknowledgement Receipt	
EFS ID:	29189875
Application Number:	15148839
International Application Number:	
Confirmation Number:	3159
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Customer Number:	133485
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Filer Authorized By:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN2
Receipt Date:	12-MAY-2017
Filing Date:	06-MAY-2016
Time Stamp:	16:00:57
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$500
RAM confirmation Number	051517INTEFSW00002792120080
Deposit Account	120080
Authorized User	Jill Sloper
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.16 (National application filing, search, and examination fees) 37 CFR 1.17 (Patent application and reexamination processing fees)	

37 CFR 1.19 (Document supply fees)

37 CFR 1.20 (Post Issuance fees)

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	IDSTRANS.PDF	28656 2f66e5f5f9432e809db8ba60c31aaaf60dc83278	no	3
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
2	Other Reference-Patent/App/Search documents	15260888.pdf	601179 c41de75afe619c3b3b25a084116843e1019856ed	no	12
Warnings:					
Information:					
3	Other Reference-Patent/App/Search documents	15284015.pdf	541339 52a151e9abe72d43e653f64da968bdea7a8a5972	no	11
Warnings:					
Information:					
4	Assignee showing of ownership per 37 CFR 3.73	AXJ_114BUSCN2_Executed_373.pdf	120360 24b4dacb26949aead80500eb6786b177c24650ff	no	2
Warnings:					
Information:					
5	Terminal Disclaimer Filed	AXJ_114BUSCN2_TD_Prior_Patent.pdf	90930 7f5f97886c5dc586df7173a1e848b0d9f490d911	no	2
Warnings:					
Information:					
6	Terminal Disclaimer Filed	AXJ_114BUSCN2_TD_Ref_Apps.pdf	102282 f2f5aa80218ad5866bc879e1f2041175edd4401b	no	2
Warnings:					

Information:					
7		AXJ_114BUSCN2_Response_and_Boone_Dec.pdf	1823354 b3d12fc5627103de2e541e3587f3558f4e79ad83	yes	24

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment/Req. Reconsideration-After Non-Final Reject	1	1
	Claims	2	2
	Applicant Arguments/Remarks Made in an Amendment	3	24

Warnings:					
Information:					
8	Fee Worksheet (SB06)	fee-info.pdf	32271 c646b9aca8b4266e549014f844c6dcb3cc71bf07	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	3340371		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
<u>New International Application Filed with the USPTO as a Receiving Office</u>
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: May 12, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/148,839

Confirmation No.: 3159

Filed: May 6, 2016

Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT (SIDS)

Dear Sir:

Pursuant to 37 C.F.R. §§ 1.56, 1.97, and 1.98, the attention of the United States Patent and Trademark Office is hereby directed to the Office Actions listed herein. It is respectfully requested that the information be expressly considered during the prosecution of the above-identified application.

Applicants provide herewith copies of Office Actions from Applications previously made of record:

Office Actions (copies enclosed)				
Examiner's Initials	Serial No.:	Date Mailed from USPTO	Docket No.	Examiner
	15/260,888	04-11-2017	AXJ-114BUSCN3	J. Rogers
	15/284,015	04-11-2017	AXJ-114BUSCN4	J. Rogers

The Examiner is requested to review the file histories of these applications, including cited references, Office Actions, Responses, etc., and is asked to contact Applicants' Attorney if the Examiner would like the Applicants to supply copies of any or all of the information included in any of these applications. For any of these applications, if Applicants' Attorney is not contacted by the Examiner with such a request, then it will be concluded that the Examiner has reviewed or will review the file content of these applications.

Applicants respectfully request that the Examiner initial the blank column next to the cited Office Actions, to indicate that the information has been considered by the Examiner. Alternatively, Applicants request that the Examiner insert the phrase, "All references considered except where lined through," on each page of the Information Disclosure Statement, along with the Examiner's initials.

The filing of this Information Disclosure Statement is not to be interpreted as a representation that the cited documents are material, that an exhaustive search has been conducted, or that no other relevant information exists. Nor shall the citation of any documents herein be construed *per se* as a representation that such document is prior art. Moreover, Applicants understand the Examiner will make an independent evaluation of the cited documents.

This Information Disclosure Statement is filed after the mailing date of a first Office Action on the merits, but before the mailing date of any of a Final Action under 37 C.F.R. § 1.113, a Notice of Allowance under 37 C.F.R. § 1.311 or an action that otherwise closes prosecution in this application (37 C.F.R. § 1.97(c)).

Samsung Bioepis Exhibit No. 1002
Page 1395

Please charge our Deposit Account No. 12-0080 in the amount of $180.00 covering the fee set forth in 37 C.F.R. § 1.17(p). The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN2.

Dated: May 12, 2017

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 217-4699 (Fax)
Attorney/Agent For Applicants

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/260,888	09/09/2016	Leonard BELL	AXJ-114BUSCN3	2558

133485 7590 04/11/2017

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
04/11/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

Office Action Summary	Application No. 15/260,888	Applicant(s) BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *1/19/2017*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-10* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-10* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

1. Claims 1-10 pending and under consideration in the instant examination.

2. The present application is being examined under the pre-AIA first to invent provisions.

In view of the Response filed 1/19/2017, the following rejections are maintained.

Response to Arguments

Claim Rejections - 35 USC § 102

3. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) *the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.*

4. Claims 1-2 and 7-10 are rejected under 35 U.S.C. 102(b) as being anticipated by Hillmen et al. (N Engl J Med 2004; 350: 552-9, IDS Ref. of 10/21/2016), as is evidenced by applicant's specification as filed at p.44 and Thomas et al. (Molecular Immunology, 33(17/18), pp.1389-1401, 1996).

With respect to base claim 1, Hillmen teaches a method for treating a human patient that suffers from paroxysmal nocturnal hemoglobinuria (PNH) by administering to the patient an effective amount of eculizumab (p. 552, "methods") which specifically binds to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b (p. 553, 3rd ¶).

While Hillmen does not specifically recite that the antibody that binds C5 comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO:4, the specification as filed at p. 44 discloses that SEQ ID NO: 2 and SEQ ID NO: 4 constitute the H and L chains for "eculizumab" and Hillmen, as noted supra, teaches administering "eculizumab". Hillmen further teaches that "eculizumab" is a recombinant humanized antibody that binds to C5 (p. 553, 3rd ¶) and cites Thomas which in turn describes in detail humanization of the clone referred to as "5G1.1" arrive at the humanized h5G1.1 which retain both the affinity and blocking functions of the murine 5g1.1 antibody (abstract). Accordingly and in absence of evidence to the contrary, the currently recited SEQ ID NO: 2 and SEQ ID NO: 4 which constitute the L and H chains of "eculizumab" are considered to be inherent properties of the "eculizumab" anti-C5 humanized monoclonal antibody taught by Hillmen.

Claim 2 is included because Hillmen teaches that the patients received eculizumab by infusion (p. 554 under “Treatment Schedule").

Claim 7 is included because Hillmen teaches that the patients had previously been diagnosed of aplastic anemia (p. 555, 1st ¶).

Claim 8 is included because Hillmen teaches that patients received infusions of eculizumab (600 mg) every week for four weeks, followed one week later by a 900 mg dose and then by a dose of 900 mg every other week through week 12 (p. 552 under “methods” and p. 554 under “treatment schedule”).

Claims 9-10 are included because Hillmen teaches that a decrease in lactate dehydrogenase began after a single dose of eculizumab (immediate decrease occurs within 1 week of administration of the antibody) in all patients and remained within or just above the normal range for the duration of the study (sustained decrease in mean levels of LDH) (p. 555, last ¶).

The reference teachings anticipate the claimed invention.

Applicant’s arguments submitted on 1/19/2017 were fully considered but were not considered persuasive.

Applicant has submitted a declaration by Dr. Leonard Bell that prior to March 15, 2007 effective filing date of the present application, the complete structure of eculizumab was not disclosed in the prior art; nor was it available to the public and that Hillmen fails to teach any part of the sequence of eculizumab (p. 4 of the Response).

This argument was not considered persuasive for the following reasons:

The Bell declaration submitted on 1/19/2017 states that clinical trials were conducted “but only under strict control and conditions of confidentiality”. However the declaration does not provide any evidence that the experiments conducted in the HILLMEN reference were under any condition of confidentiality, nor does it provide any evidence that the authors of that study were working under the direction of the present inventors.

It remains the examiner’s position that there is substantial evidence that eculizumab was in use more than a year before applicant filed for the patent.

As noted supra, 35 USC 102(b) states:

A person shall be entitled to a patent unless —

(b) the invention was patented or described in a printed publication in this or a foreign country **or in public use or on sale in this country**, more than one year prior to the date of the application for patent in the United States,

While it is true that if the antibody was unknown prior, then a reference which names it does not enable that antibody. See MPEP 2121.02 sections I and II. See also 2121.01, "the stated test is whether a reference contains an 'enabling disclosure'... ." In re Hoeksema, 399 F.2d 269, 158 USPQ 596 (CCPA 1968). The disclosure in an asserted anticipating reference must provide an enabling disclosure of the desired subject matter; mere naming or description of the subject matter is insufficient, if it cannot be produced without undue experimentation." And … "Such possession is effected if one of ordinary skill in the art could have combined the publication's description of the invention with his [or her] own knowledge to make the claimed invention." In re Donohue, 766 F.2d 531, 226 USPQ 619 (Fed. Cir. 1985)."

In the instant case, applicant's position is that the mere naming is insufficient. However, the extensive number of both patent literature and NPL documents as well as clinical trials indicates that eculizumab was widespread and well-known. As evidence of the availability and use of eculizumab the examiner refers applicant to the following documents, some of which (exhibits A-E) are part of the Request for additional Information which is also part of this OA:

A) **Exhibit A:** from ClinicalTrails.gov (studies 41-60).
B) **Exhibit B:** Clinicaltrials.gov from 6/23/2005 for NCT00098280
C) **Exhibit C:** "Safety and Effectiveness of h5G1.1-mAb for dermatomyositis" clinical Trials.gov identifier NCT00005571
D) **Exhibit D:** Kaplan 2002. Current Opinion in Investigational Drugs 3(7):1017-1023.
E) **Exhibit E:** Appel et al. Kidney International 2006 70:S45-S50, in particular the paragraph spanning pp. S48-S49 (http://www.kidney-international.org/article/S0085-2538(15)51857-7/pdf)
F) **Wang (US 2005/0271660)**, which references pexelizumab or eculizumab as an antibody therapeutic (¶s4 , claim 7) and is presumed to be enabled.
G) **Rother (WO 2005/110481)**. See for example claims 10, 44-45, 79-80, 123, 125, 130 as well as page 17 line 26 – p. 18 line 12 (noting that the antibody from US Patent 6355245 is now named eculizumab).
H) **Rother (US 2009/0028850)**. This is the publication of the national-stage entry of the '481 application above. Note that the underlying application (11/596382) contains claims to methods of using eculizumab; the office has considered these claims to be enabled. Note that the case recently was decided by the PTAB, and the Board agreed that claims drawn to administering eculizumab were obvious (and thus presumably enabled and operative) as of the filing date of that underlying application, 5/16/2005.

In re Antor Media Corp (CAFC, 2012) states that subject matter disclosed in a reference, whether it is a patent claim, other parts of a patent, or non-patent literature, is presumed to be enabling absent evidence to the contrary.

It remains the examiner's position that eculizumab was effectively in the public domain over a year prior to the filing of this application, as indicated by the preponderance of evidence.

Claim Rejections - 35 USC § 103

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained even though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

6. Claims 1-10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hillmen et al. (N Engl J Med 2004; 350: 552-9), in view of Thomas et al. (Molecular Immunology, 33(17/18), pp.1389-1401, 1996) and of Evans et al. (US 6,355,245 B1, issued 3/12/2002), and further in view of Wang (US 2005/0271660).

The prior art teachings of Hillmen are discussed supra. The prior art teachings of Thomas and the '245 patent were discussed in the prior OA.

The prior art teachings differ from the claimed invention in the recitation that the antibody is administered at a dosage level of between 5-50 mg/kg per patient per treatment as in claim 3, that the composition is in a single unit dosage form as in claim 4 and that the single unit dosage form is a 300 mg single unit dosage form as in claim 5.

The prior art teachings further differ in the recitation that the pharmaceutical composition comprises a 300 mg single-use formulation of 30 ml of a 10 mg/ml sterile, preservative free solution as in claim 6.

The ‘245 patent further teaches antibody 5G1.1, which is the same as eculizumab (discussion supra), is effective at doses of 2.2, 3, or 5 mg per kg body weight; this corresponds to 154, 210, or 350 mg of antibody for a 70kg adult human (‘245 patent, column 16 lines 10-30). Evans (the ‘245 patent) also teaches that eculizumab is therapeutically effective when as little as 210 mg are present (column 16 lines 10-15). Evans teaches that the antibodies are particularly advantageous in that they are highly active even when administered in low doses (col 8 lines 25-32), providing a reasonable expectation of success in treating PHN with a dose less than what was disclosed by Hillmen. The reference teaches that it is within the skill of an artisan, particularly a physician, to determine the optimal dose (column 18 lines 15-30). Evans teaches formulations of the anti-C5 antibodies may be formulated in a pharmaceutically effective carrier such saline, which is considered to be a "preservative free solution". (col 18 lines 30-45).

Neither Hillmen nor the ‘245 patent (Evans) teaches a 300 mg single unit dosage as recited in the claims.

Nevertheless it would have been obvious to one of ordinary skill in the art to modify the teachings of Hillmen to administer 300 mg single unit dosages. Hillmen teaches that both 600 and 900 mg dosages can be used, and since Evans patent teaches that 210 or 350 mg of antibody would be expected to be therapeutically effective, arriving at the 300 mg dosage would be routine, convenient and obvious. One of ordinary skill in the art would have a reasonable expectation of success, as Evans teaches that the antibody exerts its effect at low doses.

It is further noted, [I]t is not inventive to discover the optimum or workable ranges by routine experimentation.” In re Aller, 220 F.2d 454, 456 (CCPA 1955); see also In re Peterson, 315 F.3d 1325 (Fed. Cir. 2003). “Only if the ‘results of optimizing a variable’ are ‘unexpectedly good’ can a patent be obtained for the claimed critical range.” In re Geisler, 116 F.3d 1465, 1469 (Fed. Cir. 1997) (quoting In re Antonie, 559 F.2d 618, 620 (CCPA 1977)). “[Discovery of an optimum value of a result effective variable in a known process is ordinarily within the skill of the art.” In re Boesch, 617 F.2d 272, 276 (CCPA 1980).

Finally, **Wang** teaches that eculizumab is highly soluble, including at up to 30 mg per ml (paragraph 0171, Figure 10). Wang further teaches that pharmaceutically acceptable carriers include pyrogen-free water may be used in formulation of the antibody (¶71) (sterile, preservative free solution). Therefore the artisan of ordinary skill would have had a reasonable expectation of success in making a 10 mg / ml solution of the antibody sterile, preservative free solution.

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

Applicant's arguments filed on 1/19/2017 were fully considered but were not considered persuasive.

Applicant's arguments mirror applicant's arguments with respect to the 35 USC 102(b) rejection over Hillmen supra. Accordingly, the examiner refers applicant to the examiner's rebuttal supra.

Request for Additional Information Under 37 CFR 1.105

7. Applicant and the assignee of this application are required under 37 CFR 1.105 to provide the following information that the examiner has determined is reasonably necessary to the examination of this application.

8. In response to this requirement, please provide answers to each of the following interrogatories eliciting factual information:

(A) **Studies #41-60 in the attached Exhibit A** provide prima facie evidence that the methods now claimed were in use in this country more than a year before the application for patent was filed. Therefore for each of studies #41-60 in the attached document Exhibit A, please answer the following questions:

(i) Whether any of the trials (#s41-60) used the same sequences for eculizumab as currently claimed (i.e., SEQ ID 2 and 4).

(ii) Whether any of the researcher(s) at each of the study sites identified in (i) above for which the same sequences currently claimed was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (ii) above?

(iv) Were the researchers for those trials identified in (i) acting solely at the direction of the present inventors?

(B) **the study entitled "Safety and Effectiveness of h5G1.1-mAb for Dermatomyositis" listed on the attached Exhibit B** please answer the following questions:

(i) Whether the trial used the same sequences for eculizumab as currently claimed (i.e., SEQ ID 2 and 4).

(ii) Whether any of the researcher(s) at each the study sites was under any confidentiality agreement?

(ii) What was the nature of such confidentiality agreement in (ii) above?

(iii) Were the researchers acting solely at the direction of the present inventors?

(C) **Exhibit D: "Eculizumab Alexion –Mariana Kaplan**: please answer the following questions with respect to the study (445382) reference on p. 1018, 2nd ¶:

(i) Whether the trial used the same sequences for eculizumab as currently claimed (i.e., SEQ ID 2 and 4).

(ii) Whether any of the researcher(s) at the study site was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (i) above?

(iv) Were the researchers acting solely at the direction of the present inventors?

(D) **Exhibit E (Appel "new approaches to the treatment of glomerular diseases)**, please answer the following questions with respect to the randomized, placebo-controlled trial in 200 patients referenced at the end of p. S48:

(i) Whether the trial used the same sequences for eculizumab as currently claimed (i.e., SEQ ID 2 and 4).

(ii) Whether any of the researcher(s) at study site was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (ii) above?

(iv) Were the researchers acting solely at the direction of the present inventors?

(E) On what date was eculizumab as encompassed by the currently claimed invention (i.e., H chains SEQ ID NO: 2 and L chain SEQ ID NO: 4) first offered for sale?

9. The applicant is reminded that the reply to this requirement must be made with candor and good faith under 37 CFR 1.56. Where the applicant does not have or cannot readily obtain an item of required information, a statement that the item is unknown or cannot be readily obtained may be accepted as a complete reply to the requirement for that item.

Double Patenting

10. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *Jn re Berg,* 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *Jn re Goodman,* 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi,* 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum,* 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel,* 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321 (c) or 1.321 (d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(1)(1) - 706.02(1)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321 (b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

11. Claims 1-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-19 of **US Patent No. 9,494,601,** view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Thomas et al. (Molecular Immunology, 33 (17/18), pp. 1389-1401), Evans et al. (US 6,355,245 81, issued 3/12/2002) and Wang (US 2005/0271660)**.**

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '601 claim eculizumab (see claim 10 of the '601).

The differences between the claimed invention and ‘601 (treatment of a patient suffering from PNH and recited doses) would be obvious in view of Hillmen, Thomas, Evan and Wang as discussed supra.

12. Claims 1-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-17 of **US Patent No. 9,409,980,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Thomas et al. (Molecular Immunology, 33 (17/18), pp. 1389-1401), Evans et al. (US 6,355,245 81, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '980 claim eculizumab (see claim 5 of the '980).

The differences between the claimed invention and ‘980 (treatment of a patient suffering from PNH and recited doses) would be obvious in view of Hillmen, Thomas, Evan and Wang as discussed supra.

13. Claims 1-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-14 of **US Patent No. 9,556,263,** view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Thomas et al. (Molecular Immunology, 33 (17/18), pp. 1389-1401), Evans et al. (US 6,355,245 81, issued 3/12/2002) and Wang (US 2005/0271660)**.**

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '263 claim eculizumab (see claims 2 and 4 of the '263).

The differences between the claimed invention and ‘263 (treatment of a patient suffering from PNH and recited doses) would be obvious in view of Hillmen, Thomas, Evan and Wang as discussed supra.

14. No claim is allowed.

15. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270-5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Daniel Kolker** can be reached on (571) 272-3181. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you

have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

March 30, 2017 /JAMES ROGERS/
Art Unit 1644

/DANIEL E. KOLKER/

Supervisory Patent Examiner, Art Unit 1644

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/284,015	10/03/2016	Leonard BELL	AXJ-114BUSCN4	1040

133485 7590 04/11/2017

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
04/11/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

Office Action Summary	Application No. 15/284,015	Applicant(s) BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *2/1/2017*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *2/1/2017*.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

1. Claims 1 is pending and under consideration in the instant examination and reads On an antibody that binds C5 comprising a H chain SEQ ID NO: 2 and a L chain SEQ ID NO: 4.

2. Applicant's two IDSs submitted on 2/1/2017 are acknowledged and have been considered.

3. Applicant has overcome the outstanding double patenting rejection from the prior OA in light of applicant's approved TDs over 15/148,839 and 15/260,888, filed 2/1/2017.

Claim Rejections - 35 USC§ 102

4. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless -
(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

5. Claim 1 is rejected under 35 U.S.C. 102(b) as being anticipated by Hillmen et al. (N Engl J Med 2004; 350: 552-9, IDS Ref. of 10/21/2016), as is evidenced by applicant's specification as filed at p.44 and Thomas et al. (Molecular Immunology, 33(17/18), pp.1389-1401, 1996).

Hillmen teaches a method for treating a human patient that suffers from paroxysmal nocturnal hemoglobinuria (PNH) by administering to the patient an effective amount of eculizumab (p. 552, "methods") which specifically binds to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b (p. 553, 3rd ¶).

While Hillmen does not specifically recite that the antibody that binds CS comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID N0:4, the specification as filed at p. 44 discloses that SEQ ID NO: 2 and SEQ ID NO: 4 constitute the H and L chains for "eculizumab" and Hillmen, as noted supra, teaches administering "eculizumab". Hillmen further teaches that "eculizumab" is a recombinant humanized antibody that binds to C5 (p. 553, 3rd paragraph) and cites Thomas which in turn describes in detail humanization of the clone referred to as "5G1 .1" arrive at the humanized h5G1 .1 which retain both the affinity and blocking functions of the murine 5g1 .1 antibody (abstract). Accordingly and in absence of evidence to the contrary, the currently recited SEQ ID NO: 2 and SEQ ID NO: 4 which constitute the Land H chains of "eculizumab" are considered to be inherent properties of the "eculizumab" anti-C5 humanized monoclonal antibody taught by Hillmen.

The reference teachings anticipate the claimed invention.

To the extent that applicant will argue that Hillmen names eculizumab but does not teach the precise sequences being currently claimed, please note the following:
35 USC 102(b) states:

A person shall be entitled to a patent unless -

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States,

While it is true that if the antibody was unknown prior, then a reference which names it does not enable that antibody. See MPEP 2121.02 sections I and II. See also 2121.01, "the stated test is whether a reference contains an 'enabling disclosure' .... " In re Hoeksema, 399 F.2d 269, 158 USPQ 596 (CCPA 1968). The disclosure in an asserted anticipating reference must provide an enabling disclosure of the desired subject matter; mere naming or description of the subject matter is insufficient, if it cannot be produced without undue experimentation." And ... "Such possession is effected if one of ordinary skill in the art could have combined the publication's description of the invention with his [or her] own knowledge to make the claimed invention." In re Donohue, 766 F.2d 531, 226 USPQ 619 (Fed. Cir. 1985)."

In the instant case, applicant's position is that the mere naming is insufficient. However, the extensive number of both patent literature and NPL documents as well as clinical trials indicates that eculizumab was widespread and well-known. As evidence of the availability and use of eculizumab the examiner refers applicant to the following documents, some of which are part of the Request for additional Information which is also part of this OA:

A) Exhibit A: from clinicalTrials.govClinicaltrials.gov (studies 41-60).

B) Exhibit B: (printout for NCT00005571), in particular page 3 indicating that the trial was ongoing in Bethesda MD, and bottom of page 1 indicating that the drug used was eculizumab.

C) Exhibit C: Clinicaltrials.gov from 6/23/2005 for NCT00098280

D) Exhibit D: Kaplan 2002. Current opinion in Investigation Drugs 3(7): 1017-1023

E) Exhibit E: Appel et al. Kidney International 2006 70:S45-S50, in particular the paragraph spanning pp. S48-S49.

F) Wang (US 2005/0271660). The reference is a US Patent Application Publication claiming methods of using eculizumab (see claim 7), and is presumed to be enabled.

G) Rother (WO 2005/110481). See for example claims 10, 44-45, 79-80, 123, 125, 130 as well as page 17 line 26 - p. 18 line 12 (noting that the antibody from US Patent 6355245 is now named eculizumab).

H) Rother (US 2009/0028850). This is the publication of the national-stage entry of the '481 application above. Note that the underlying application (11/596382) contains claims to methods of using eculizumab; the office has considered these claims to be enabled. Note that the case recently was decided by the PTAB, and the Board agreed that claims drawn to administering eculizumab were obvious (and thus presumably enabled and operative) as of the filing date of that underlying application, 5/16/2005.

In re Antor Media Corp (CAFC, 2012) states that subject matter disclosed in a reference, whether it is a patent claim, other parts of a patent, or non-patent literature, is presumed to be enabling absent evidence to the contrary.

Accordingly, it is the examiner's position that eculizumab was effectively in the public domain.

6. Claim 1 is rejected under 35 U.S.C. 102(b) as being anticipated by **Appel et al.** (Kidney International 70, S45-S50, 2006).

Appel teaches that eculizumab is a humanized monoclonal antibody that prevents the activation of complement component C5. Appel further teaches a randomized trial in 200 patients with eculizumab (S48 and spanning to S49)).

While Appel does not specifically recite that the antibody that binds C5 comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID N0:4, in absence of evidence to the contrary, the currently recited SEQ ID NO: 2 and SEQ ID NO: 4 which constitute the Land H chains of "eculizumab" are considered to be inherent properties of the "eculizumab" anti-C5 humanized monoclonal antibody taught by Appel.

The reference teachings anticipate the claimed invention.

7. Claim 1 is rejected under 35 U.S.C. 102(b) as being anticipated by **Wang** (US2005/0271660).

Wang teaches an eculizumab that inhibits activation of the complement system (¶s11, 67, 171).

While Wang does not specifically recite that the antibody that binds C5 comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID N0:4, in absence of evidence to the contrary, the currently recited SEQ ID NO: 2 and SEQ ID NO: 4 which constitute the Land H chains of "eculizumab" are considered to be inherent properties of the "eculizumab" anti-C5 humanized monoclonal antibody taught by Wang.

The reference teachings anticipate the claimed invention. Note that the reference claims eculizumab.

Request for Additional Information Under 37 CFR 1.105

8. Applicant and the assignee of this application are required under 37 CFR 1.105 to provide the following information that the examiner has determined is reasonably necessary to the examination of this application.

9. In response to this requirement, please provide answers to each of the following interrogatories eliciting factual information:

(a) Studies #41-60 in the attached Exhibit A provide prima facie evidence that the methods now claimed were in use in this country more than a year before the document Exhibit A, please answer the following questions:

(i) Which is any of these studies disclose the same antibody sequences for eculizumab encompassed by the claims of the current application (i.e., SEQ ID NO: 2 and SEQ ID NO: 4)?

(ii) Whether any of the researcher(s) at each of the study sites for which eculizumab covered by the current claims in (i) above was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (ii) above?

(iv) Were the researchers in (i) above acting solely at the direction of the present inventors?

(b) the study entitled "Safety and Effectiveness of h5G 1.1-mAb for Dermatomyositis" listed on the **attached Exhibit B** please answer the following questions:

(i) Does this study disclose the same antibody sequences for eculizumab encompassed by the claims of the current application (i.e., SEQ ID NO: 2 and SEQ ID: 4)?

(ii) Whether any of the researcher(s) at the study site for any study identified in (i) above was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (ii) above?

(iv) Were the researchers for any study identified in (i) above acting solely at the direction of the present inventors?

(C) For the study entitled "View of NCT00098280 on 2005_06_23" attached as exhibit C, please answer the following questions:

(i) Does this study disclose the same antibody sequences for eculizumab encompassed by the claims of the current application (i.e., SEQ ID NO: 2 and SEQ ID: 4)?

(ii) Whether any of the researcher(s) the study site identified in (i) above was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (ii) above?

(iv) Were the researchers for any study identified in (i) above acting solely at the direction of the present inventors?

(D) Exhibit D: "Eculizumab Alexion –Mariana Kaplan: please answer the following questions with respect to the study (445382) reference on p. 1018, 2nd ¶:

(i) Whether the trial used the same sequences for eculizumab as currently claimed (i.e., SEQ ID 2 and 4).

(ii) Whether any of the researcher(s) at the study site was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (i) above?

(iv) Were the researchers acting solely at the direction of the present inventors?

(E) Exhibit E (Appel "new approaches to the treatment of glomerular diseases), please answer the following questions with respect to the randomized, placebo-controlled trial in 200 patients referenced at the end of p. S48:

(i) Whether the trial used the same sequences for eculizumab as currently claimed (i.e., SEQ ID 2 and 4).

(ii) Whether any of the researcher(s) at study site was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (ii) above?

(iv) Were the researchers acting solely at the direction of the present inventors?

(F) On what date was eculizumab which is encompassed by the current claimed (i.e., SEQ ID NOs: 2 and 4) first offered for sale?

10. The applicant is reminded that the reply to this requirement must be made with candor and good faith under 37 CFR 1.56. Where the applicant does not have or cannot readily obtain an item of required information, a statement that the item is unknown or cannot be readily obtained may be accepted as a complete reply to the requirement for that item.

Double Patenting

11. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *Jn re Berg,* 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *Jn re Goodman,* 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi,* 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum,* 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel,* 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321 (c) or 1.321 (d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(1)(1) - 706.02(1)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321 (b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/e TD-info-1.jsp.

12. Claim 1 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 7, 9, and 10 of **US Patent No. 9,494,601.**

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '601 claim eculizumab (see claim 10 of the '601)

13. Claim 1 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-18 of **US Patent No. 8,999,340.**

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '340 claim eculizumab (see claim

9 of the '34 patent0).

14. Claim 1 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-17 of **US Patent No. 9,409,980.**

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '980 claim eculizumab (see claim 5 of the '980).

15. Claim 1 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-14 of **US Patent No. 9,556,263.**

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '263 claim eculizumab (see claims 2 and 4 of the '263)

16. Claim 1 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-22 of **US Patent No. 9,352,035.**

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '035 claim eculizumab (see claim 1 of the '035).

17. Claim 1 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-15 of **US Patent No. 9,415,102.**

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '102 claim eculizumab (see for example claims 4 and 15 of the '102).

18. Claim 1 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-17 of **US Patent No. 9,409,980**.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '980 claim eculizumab (see claims 3-4 of the '980).

19. Claim 1 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4 of **US Patent No. 9,447,176**.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '176 claim eculizumab (see claim 1 of the '176).

20. Claim 1 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-5, 7, and 9-16 of **US Patent No. 9,388,235**.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '235 claim eculizumab (see claim 5 of the '235).

21. Claim 1 is provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 15-17, 59 of **US Patent Application No: 11/127,438**.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '438 claim eculizumab (note the specification of the ‘438 application clearly encompasses eculizumab as one of the preferred antibodies).

22. Claim 1 is provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4 of **US Patent Application No: 15/243,386.**

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '386 claim eculizumab (see claim 1 of the '386).

23. Claim 1 is provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4 of **US Patent Application No: 15/243,290.**

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '290 claim eculizumab (see claim 1 of the '290).

24. Claim 1 is provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 94 and 97-98 of **US Patent Application No: 15/282,464**.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.
Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '464 claim eculizumab (see claim 94 of the '464)

25. Claim 1 is provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 129 – 138 of **US Patent Application No: 15/148,491**.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '491 claim eculizumab (see claim 129 of the '491)

27. No claim is allowed.

28. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270-5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel Kolker can be reached on (571) 272-3181. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

March 21, 2017

/JAMES ROGERS/

/DANIEL E. KOLKER/

Supervisory Patent Examiner, Art Unit 1644

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: Alexion Pharmaceuticals, Inc.

Application No./Patent No.: 15/148,839 Filed/Issue Date: May 6, 2016

Titled: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Alexion Pharmaceuticals, Inc. (Name of Assignee) , a Corporation (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. [X] The assignee of the entire right, title, and interest.

2. [ ] An assignee of less than the entire right, title, and interest (check applicable box):

   [ ] The extent (by percentage) of its ownership interest is _____ %). Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

   [ ] There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. [ ] The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. [ ] The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

A. [ ] An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____ , Frame _____ , or for which a copy thereof is attached.

B. [X] A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: Leonard Bell To: Alexion Pharmaceuticals, Inc.
   The document was recorded in the United States Patent and Trademark Office at
   Reel 038520 , Frame 0957 , or for which a copy thereof is attached.

2. From: Russell P. Rother To: Alexion Pharmaceuticals, Inc.
   The document was recorded in the United States Patent and Trademark Office at
   Reel 038520 , Frame 0957 , or for which a copy thereof is attached.

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

3. From: Mark J. Evans To: Alexion Pharmaceuticals, Inc.
The document was recorded in the United States Patent and Trademark Office at
Reel 041505 , Frame 0468 , or for which a copy thereof is attached.

4. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached.

5. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached.

6. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☒ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

Todd Spalding
Signature

May 11, 2017
Date

Todd Spalding
Printed or Typed Name

Vice President, Head of Intellectual Property
Title or Registration Number

PTO/AIA/26 (04-14)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT	Docket Number (Optional) AXJ-114BUSCN2

In re Application of: Leonard BELL *et al.*

Application No.: 15/148,839-Conf. #3159

Filed: May 6, 2016

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

The applicant, Alexion Pharmaceuticals, Inc., owner of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of **prior patent** No. (see attached) as the term of said **prior patent** is presently shortened by any terminal disclaimer. The applicant hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the **prior patent** are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the applicant does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the **prior patent**, "as the term of said **prior patent** is presently shortened by any terminal disclaimer," in the event that said **prior patent** later:

expires for failure to pay a maintenance fee;
is held unenforceable;
is found invalid by a court of competent jurisdiction;
is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321;
has all claims canceled by a reexamination certificate;
is reissued; or
is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

Check either box 1 or 2 below, if appropriate.

1. [X] The undersigned is the applicant. If the applicant is an assignee, the undersigned is authorized to act on behalf of the assignee.

I hereby acknowledge that any willful false statements made are punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

2. [ ] The undersigned is an attorney or agent of record. Reg. No. _____

Todd Spalding — Signature

May 11 2017 — Date

Todd Spalding — Typed or printed name

Vice President, Head of Intellectual Property — Title

_____ — Telephone Number

[X] Terminal disclaimer fee under 37 CFR 1.20(d) included.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

SUPPLEMENTAL SHEET FOR TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT	Docket Number (Optional) AXJ-114BUSCN2

Prior patent Nos. applicable to this terminal disclaimer (referenced on first page):

9,494,601
8,999,340
9,409,980
9,556,263
9,352,035
9,415,102
9,447,176
9,388,235

PTO/AIA/25 (04-13)
Approved for use through 04/30/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING "REFERENCE" APPLICATION	Docket Number (Optional) AXJ-114BUSCN2

In re Application of: Leonard BELL *et al.*

Application No.: 15/148,839-Conf. #3159

Filed: May 6, 2016

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

The applicant, Alexion Pharmaceuticals, Inc., owner of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of any patent granted on pending **reference** Application Number (see attached), filed, _____, as the term of any patent granted on said **reference** application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending **reference** application. The applicant hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the **reference** application are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the applicant does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term of any patent granted on said **reference** application, "as the term of any patent granted on said **reference** application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending **reference** application," in the event that: any such patent granted on the pending **reference** application expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.

Check either box 1 or 2 below, if appropriate.

1. [X] The undersigned is the applicant. If the applicant is an assignee, the undersigned is authorized to act on behalf of the assignee.

I hereby acknowledge that any willful false statements made are punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

2. [ ] The undersigned is an attorney or agent of record. Reg. No. _____

Todd Spalding — Signature — May 11, 2017 — Date

Todd Spalding — Typed or printed name

Vice President, Head of Intellectual Property — Title

_____ — Telephone Number

[X] Terminal disclaimer fee under 37 CFR 1.20(d) is included.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

SUPPLEMENTAL SHEET FOR TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING "REFERENCE" APPLICATION	Docket Number (Optional) AXJ-114BUSCN2

Reference application Nos. applicable to this terminal disclaimer (referenced on first page):

15/243,290 (filed August 22, 2016)

15/282,464 (filed September 30, 2016)

15/148,491 (filed May 6, 2016)

Application Number	Application/Control No.	Applicant(s)/Patent under Reexamination	
	15/148,839	BELL ET AL.	

Document Code - DISQ	Internal Document – DO NOT MAIL

TERMINAL DISCLAIMER	☒ APPROVED	☐ DISAPPROVED
Date Filed : 12 May, 2017	This patent is subject to a Terminal Disclaimer	

Approved/Disapproved by:

/CRYSTAL QUEEN/

Technology Center: PLRC

Telephone: _____

2 TDS APPROVED.

U.S. Patent and Trademark Office

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/148,839	Filing Date 05/06/2016	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	05/12/2017	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 3	Minus	** 20	= 0	X $80 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $420 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	**0**

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

SLIE
HELENA PAYTON

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

133485 7590 06/07/2017

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER
ROGERS, JAMES L

ART UNIT	PAPER NUMBER
1644	

DATE MAILED: 06/07/2017

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/148,839	05/06/2016	Leonard BELL	AXJ-114BUSCN2	3159

TITLE OF INVENTION: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	09/07/2017

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

133485 7590 06/07/2017
Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/148,839	05/06/2016	Leonard BELL	AXJ-114BUSCN2	3159

TITLE OF INVENTION: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	09/07/2017

EXAMINER	ART UNIT	CLASS-SUBCLASS
ROGERS, JAMES L	1644	424-133100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:
- ❑ Issue Fee
- ❑ Publication Fee (No small entity discount permitted)
- ❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ❑ A check is enclosed.
- ❑ Payment by credit card. Form PTO-2038 is attached.
- ❑ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ❑ Applicant certifying micro entity status. See 37 CFR 1.29
  NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
- ❑ Applicant asserting small entity status. See 37 CFR 1.27
  NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
- ❑ Applicant changing to regular undiscounted fee status.
  NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Samsung Bioepis Exhibit No. 1002
Page 1429

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/148,839	05/06/2016	Leonard BELL	AXJ-114BUSCN2	3159

133485 7590 06/07/2017

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER
ROGERS, JAMES L

ART UNIT	PAPER NUMBER
1644	

DATE MAILED: 06/07/2017

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

PTOL-85 (Rev. 02/11)

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 15/148,839	Applicant(s) BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *5/12/2017*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *1-3*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:
   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 5/12/2017
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
5. ☒ Examiner's Amendment/Comment
6. ☒ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/JAMES ROGERS/ Examiner, Art Unit 1644	/DANIEL E. KOLKER/ Supervisory Patent Examiner, Art Unit 1644

DETAILED ACTION

1. The present application is being examined under the pre-AIA first to invent provisions.

2. Applicant's Response, filed 5/12/2017, is acknowledged.

3. Applicant's IDS, filed 5/12/2017, is acknowledged and has been considered.

REASONS FOR ALLOWANCE

4. The following is an Examiner's Statement of Reasons for Allowance:

The claims are drawn to a pharmaceutical composition for use in treating a patient afflicted with paroxysmal nocturnal hemoglobinuria (PNH) where the composition is a sterile, preservative free, 300 mg single use dosage form comprising 30 ml of a 10 mg/ml antibody solution and wherein the antibody comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4.

Applicant's arguments in the Response of 5/12/2017 to the outstanding Non-final OA of 4/4/2017 which included rejections under 35 USC 102, 35 USC 103 and double patenting rejections as well as a Request for Additional Information Under 37 CFR 1.105 in conjunction with the Declaration Pursuant to 37 CFR 1.132 by Dr. Laural Boone, dated May 11, 2017, is persuasive to overcome all of the rejections for the following reasons:

(a) None of the applied references in the rejections recite using an antibody which comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4 as currently recited and one of skill in the art would not have been easily guided to making antibodies with these recited sequences.

(b) Applicant's Response to the Request for Additional Information is considered sufficient and persuasive to conclude that each of the clinical trials conducted by applicant referenced in the Request did not constitute "public use" under 35 USC 102(b) of the claimed invention as they were not accessible to the public. As applicant notes in the response, factors which are used to determine if an invention is accessible to the public are set out by the Federal Circuit in *Dey, L.P. v. Sunovion Pharmaceuticals, Inc.*, (No. 2012-1428, Fed. Cir. May 20, 2013). The factual circumstances cited by applicant considered by the examiner as being most persuasive to negate any determination that the referenced clinical trials constituted "public use" is the use by applicant of confidentiality provisions (see p. 11 of the response for the 16 clinical studies subject to interrogatories A-F of the Request and pp. 2-3 and p.5 (including sample confidentiality provision" of the Declaration) for the studies and the fact that neither doctors nor

patients had any knowledge of the currently claimed sequences of the antibody used in the studies (see p. 5 of the Response). Other factual evidence presented by applicant includes the declaration by Laural S. Boone that strict control over the eculizumab drug was exercised for each clinical trial referenced (see p. 6 of the Declaration).

(c) The Double Patenting rejections have been overcome in light of applicant's approved TDs of 5/12/2017 over the '601, '340, '980, '263, '035, '102, '176 and '235 patents and '290, 464 and 491' Patent Applications.

As to the remaining two applications (15/243,386 and 11/127,438), the '386 application is now abandoned. The '438 application, as noted by applicant in the Response, recites an aerosol composition comprising an antibody which does not include the currently claimed sequences.

Accordingly, claims 1-3, are deemed allowable.

5. Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

6. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270-5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Daniel E. Kolker** can be reached on (571) 272-3181. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

May 19, 2017

/JAMES ROGERS/
Examiner, Art Unit 1644

/DANIEL E. KOLKER/
Supervisory Patent Examiner, Art Unit 1644

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: May 6, 2016
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: Not Yet Assigned

Confirmation No.: N/A

Filed: Concurrently Herewith

Art Unit: N/A

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: Not Yet Assigned

INFORMATION DISCLOSURE STATEMENT (IDS)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In compliance with 37 C.F.R. § 1.56, 1.97 and 1.98, the attention of the Patent and Trademark Office is hereby directed to the documents listed on the attached PTO/SB/08.

For the Examiner's convenience in reviewing this continuation application, Applicant submits a consolidated PTO Form SB/08, listing all references cited during the prosecution of the parent application. The present application is a continuation of U.S. Application No. 13/426,973, filed March 22, 2012 (Atty. Docket No. AXJ-114BUSCN). In accordance with 37 C.F.R. §1.98(d), copies of the references previously cited by or submitted to the Office in the parent application are not enclosed, but will be provided upon request. In accordance with 37 C.F.R. § 1.98(a)(2)(i)-(iv), Applicant submits herewith copies of a non-patent literature references.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

It is respectfully requested that the documents listed on the PTO/SB/08 be expressly considered by the Examiner during the prosecution of this application, and that the documents be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

Applicants call to the attention of the Examiner the following Applications and Office Actions cited therein:

Applications				
Examiner's Initials	*Serial No.*	*Filing Date*	*First Named Inventor*	*Docket No.*
	13/426,973	03-22-2012	Leonard Bell	AXJ-114BUSCN
	12/225,040	05-13-2009	Leonard Bell	AXJ-114BUS
	12/621,792	11-19-2009	Leonard Bell	AXJ-114DV
	10/771,552	02-03-2004	Leonard Bell	AXJ-114RCE
	11/050,543	02-03-2005	Leonard Bell	AXJ-114CPRCE
	11/595,118	11-08-2006	Leonard Bell	AXJ-114CP2
	12/902,617	10-12-2010	Leonard Bell	AXJ-114CPCN
	13/335,518	12-22-2011	Leonard Bell	AXJ-114CPCN2RCE

Office Actions (copies enclosed)			
Examiner's Initials	Serial No.	Date Mailed from USPTO	Examiner
	13/426,973	01-27-2014	J. Rogers
	13/426,973	05-31-2013	J. Rogers
	13/426,973	08-20-2012	J. Rogers
	12/225,040	09-22-2011	J. Rogers
	12/225,040	06-01-2011	J. Rogers
	12/225,040	04-11-2011	J. Rogers
	12/621,792	09-30-2010	P. Gambel
	10/771,552	12-22-2008	F. Vandervegt
	10/771,552	03-21-2008	F. Vandervegt
	10/771,552	08-10-2007	F. Vandervegt
	10/771,552	02-26-2007	F. Vandervegt
	10/771,552	09-26-2006	F. Vandervegt
	11/050,543	07-09-2010	P. Gambel
	11/050,543	04-02-2008	F. Vandervegt
	11/050,543	10-10-2007	F. Vandervegt
	11/050,543	06-11-2007	F. Vandervegt
	11/595,118	01-21-2011	P. Gambel
	11/595,118	10-05-2010	P. Gambel

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

	11/595,118	07-07-2010	P. Gambel
	11/595,118	04-03-2009	F. Vandervegt
	12/902,617	06-23-2011	P. Gambel
	12/902,617	04-04-2011	P. Gambel
	13/335,518	09-12-2014	P. Gambel
	13/335,518	02-06-2014	P. Gambel
	13/335,518	11-07-2013	P. Gambel

/JAMES L ROGERS/ 08/08/2016

The filing of this Information Disclosure Statement is not to be interpreted as a representation that the cited documents are material, that an exhaustive search has been conducted, or that no other relevant information exists. Nor shall the citation of any documents herein be construed *per se* as a representation that such document is prior art. Moreover, Applicant understands the Examiner will make an independent evaluation of the cited documents.

This Information Disclosure Statement accompanies the new patent application submitted herewith. Accordingly, no fees are due with this submission (37 C.F.R § 1.97(b)(3)). However, the Director is hereby authorized to charge any fee deficiency which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN2.

Dated: May 6, 2016

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY &
SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 742-4214 (Fax)
Attorney/Agent For Applicant

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (03-15)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Not for submission under 37 CFR 1.99)

Application Number	15/148839
Filing Date	
First Named Inventor	Leonard BELL
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	AXJ-114BUSCN2

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	4686100		1987-08-11	Raffin et al.	
	2	5135916		1992-08-04	Sims et al.	
	3	5660825		1997-08-26	Sims et al.	
	4	6355245		2002-03-12	Evans et al.	
	5	7361339		2008-04-22	Bell	
	6	7482435		2009-01-27	Bowdish et al.	

If you wish to add additional U.S. Patent citation information please click the Add button. Add

U.S.PATENT APPLICATION PUBLICATIONS Remove

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear

EFS Web 2.1.17

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15/148839
	Filing Date	
	First Named Inventor	Leonard BELL
	Art Unit	N/A
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	AXJ-114BUSCN2

	1	20030049683	A1	2003-03-13	Bowdish et al.	
	2	20050191298	A1	2005-09-01	Bell et al.	
	3	20050221382	A1	2005-10-06	Rother	
	4	20050169921	A1	2005-08-04	Bell et al.	
	5	20050271660	A1	2005-12-08	Wang	
	6	20070116710	A1	2007-05-24	Bell et al.	
	7	20090220508	A1	2009-09-03	Bell et al.	
	8	20100068202	A1	2010-03-18	Bell et al.	
	9	20110086040	A1	2011-04-14	Bell et al.	
	10	20120308559	A1	2012-12-06	Bell et al.	
	11	20120237515	A1	2012-09-20	Bell et al.	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15/148839
	Filing Date	
	First Named Inventor	Leonard BELL
	Art Unit	N/A
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	AXJ-114BUSCN2

If you wish to add additional U.S. Published Application citation information please click the Add button. Add

FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2]	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	0411306	EP	A1	1991-02-06	Goetze, Otto Prof. Dr. Med		
	2	2004/029207	WO	A2	2004-04-08	Xencor Inc		
	3	2005074607	WO	A2	2005-08-18	Alexion Pharmaceuticals, Inc		

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	Alexion Announces Presentation of Preliminary Results from Phase I Pilot Study of Eculizumab Treatment of Patients with Paroxysmal Nocturnal Hemoglobinuria"; [online]; Alexion Pharmaceuticals: News; [Retrieved on 2003-06-18]. Retrieved from the world wide web at alexionpharmaceuticals.com/news/sub press.cfm?prid=242&selectyear=2002.	
	2	Alexion Announces Results of Clinical Trial in Paroxysmal Nocturnal Hemoglobinuira Presented at the American Society of Hematology Annual Meeting"; [online]; Alexion Pharmaceuticals, Inc.; [Retrieved on 2003-06-17]; Retrieved from the world wide web at noonanrusso.com/news/view newsitem.aspx?ItemID=382.	
	3	Alexion Pharmaceuticals: Complement Inhibitors; [online]; [Retrieved on 2003-06-17]; Retrieved from the world wide web at alexionpharm.com/techplat/complement.cfm.	
	4	Alexion Pharmaceuticals Signs Agreement with Lonza Biologics for Commercial Manufacturing; [online]; Alexion Pharmaceuitcals Inc.; [Retrieved on 2003-06-17]; Retrieved from the world wide web at noonanrusso.com/news/view newsitem.aspx?ItemID=394.	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15/148839
	Filing Date	
	First Named Inventor	Leonard BELL
	Art Unit	N/A
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	AXJ-114BUSCN2

	5	Alexion receives FDA, European approval for orphan drug status of new treatment for paroxysmal nocturnal hemoglobinuria, TRANSPLANT NEWS, 01-15-2004, XP002497241;Retrieved from the world wide web at findarticles.com/p/articles/mi mOYUG/is 1 14/ai 17208612 [retrieved on 09-25-2008]	
	6	Alexion Reports Presentation of Membranous Nephritis Clinical Trails"; [online]; Alexion Pharmaceuticals: News; [Retrieved on 2003-06-17]. Retrieved from the world wide web at alexionpharm.com/news/sub press.cfm?prid=241 &selectyear=2002.	
	7	Araten, et al.," High incidence of thrombosis in African-American and Latin-American patients with paroxysmal nocturnal haemoglobinuria," Thromb Haemost, vol. 93:88-91 (2005)	
	8	Audebert et al., "Cerebral ischemic infarction in paroxysmal nocturnal hemoglobinuria," J. Neurol., vol .252:1379-2565 (2005).	
	9	Brodsky, "New Insights into paroxysmal nocturnal hemoglobinuria," Hematology 516:24-28 (2006)	
	10	Cella, D et al., "Linking Outcomes Management to Quality-of-Life Measurement," Oncology," Williston Park, vol. 11 (11A): 232-235 (1997).	
	11	Clague et al, "A low-hemolysis blood aspirator conserves blood during surgery," Biomed. Insrum. Technol. , vol. 29 (5):419-424 (1995)	
	12	Clinics, Biotechnology News, vol. 24(30):10 (2004).	
	13	Collard et al., "Endothelial nuclear factor-KB translocation and vascular cell adhesion molecule- 1 induction by complement: inhibition with anti-human C5 therapy or cGMP analogues," Art. Thromb. Vas., vol. 19:2623-2629 (1999).	
	14	SIERRA G. et al., "Vaccine against group B Neisseia meningitidis: Protection trial and mass vaccination results in Cuba", NIPH ANNALS 1991 NO, vol. 14(2):195-210, (1991) ISSN: 0332-5652	
	15	CORVINI M. et al., "Complement C7 deficiency presenting as recurrent aseptic meningitis," Database accession no. NLM15328683, 1 page (2004) DATABASE MEDLINE [Online] US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; October 1992 (1992-10)	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15/148839
	Filing Date	
	First Named Inventor	Leonard BELL
	Art Unit	N/A
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	AXJ-114BUSCN2

	16	MORRIS J T ET AL: "Recurrence of neisserial meningococcemia due to deficiency of terminal complement component," Database accession no. NLM1411724, DATABASE MEDLINE [Online] US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; October 1992 (1992-10)	
	17	Figueroa and Densen, "Infectious Diseases Associated with Complement Deficiencies," Clinical Microbiology Reviews, vol. 4(3): 359-395 (1991).	
	18	Fitch et al., "Pharmacology and biological efficacy of a recombinant, humanized, single-chain antibody C5 complement inhibitor in patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass," Circulation 100 (25):2499-2506 (1999).	
	19	Garba and Ubom, "Total serum lactate dehydrogenase activity in acute Plasmodium falciparum malaria infection," Singapore Medical J. 46(11):632-634 (2005).	
	20	Garcia et al. "Effect of preservatives on IgG aggregation, complement-activating effect and hypotensive activity of hourse; polyvalent antivenom used in snakebite envenomation," Biologicals, vol. 30:143-151(2002)	
	21	Genes and Disease; [online]; Paroxysmal nocturnal hemoglobinuria; [Retrieved on 2003-06017]. Retrieved from the world wide web at ncbi.nlm.nih.gov/disease/PNH.html	
	22	Hall et al., "Primary prophylaxis with warfarin prevents thrombosis in paroxysmal nocturnal hemoglobinuria (PNH)," Blood, vol. 102 3587-3591 (2003)	
	23	Harding, J. "Eculizumab," Drugs of the Future, Pro US Science, ES, vol. 29(7): 673-676, XP008113000 (2004).	
	24	Hill et al., "Improvement in the symptoms of smooth muscle dystonia during eculizumab therapy in paroxysmal nocturnal hemoglobinuria," Haematology, 90(12): Abstract #ECR40; (2005)	
	25	Hill et al., "Sustained Control of Hemolysis and Symptoms and Reduced Transfusion Requirements over a Period of 2 Years in Paroxysmal Nocturnal Hemoglobinuria (PNH) with Eculizumab Therapy," Blood, vol. 104:Abstract 2823 (2004) with ASH poster final 2004.ppt.	
	26	Hill et al., "Sustained response and long-term safety of eculizumab in paroxysmal nocturnal hemoglobinuria," Blood, vol. 106: 2559-2565 (2005).	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15/148839
	Filing Date	
	First Named Inventor	Leonard BELL
	Art Unit	N/A
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	AXJ-114BUSCN2

	27	Hill, et al., "Protection of erythrocytes from human complement-mediated lysis by membranetargeted recombinant soluble CD59: a new approach to PNH therapy," Blood 107(5):2131-2137 (2006).	
	28	Hillmen et al. "Effect of Eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal Hemoglobinuria," N Engl J Med, vol. 350(6):552-559 (2004)	
	29	Hill et al. "Improvement in the symptoms of smooth muscle dystonia during eculizumab therapy in paroxysmal nocturnal; hemoglobinuria" Haematologica, vol. 90(online): 111-113 (2005)	
	30	Hill, et al., "Nitric oxide consumption and pulmonary hypertension in patients with paroxysmal nocturnal hemoglobinuria," Blood 106(11):Abstract 1046 (2005) XP002482085 - 47th Annual Meeting Of the American Society of Hematology, December 10-13, 2005.	
	31	Hill, "Eculizumab, for the treatment of paroxysmal nocturnal hemglobinuria," Clin. Adv. In Hemal. & Onc., XP0015384263 (11):849-850 (2005) .	
	32	Hillman et al., "Effect of the complement inhibitor eculizumab on thromboembolism in patients with paroxysmal nocturnal hemoglobinuria," Blood 110(12):4123-4128 (2007).	
	33	Hillmen, et al., "Eculizumab, a C5 Complement-blocking Antibody, Abolishes Hemolysis and Renders Hemolytic Patients with Paroxysmal Nocturnal Hemoglobinuria (PNH) Transfusion Independent," 44th ASH Annual Meeting, Blood 100 (11) (Abstract No. 154) (2002).	
	34	Meyers, et al., "Management Issues in Paroxysmal Nocturnal Hemoglobinuria," International Journal of Hematology, 77:125-132 (2003).	
	35	Hillmen, et al., "The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria," N Engl J Med. ., vol. 355: 1233-1243 (2006).	
	36	Hillmen, et al., "The terminal complement inhibitor eculizumab reduces thrombosis in patients with paroxysmal nocturnal hemoglobinuria," Blood 108(11):Abstract 123 (2006) XP002482084. 48th Annual Meeting of The American Society of Hematology. December 9-12, 2006.	
	37	Hillmen, et al., "Eculizumab, a C5 complement blocking antibody, is the first therapy to reduce transfusion requirements in patients with paroxysmal nocturnal haemoglobinuria (PNH); British Journal of Haematology, vol.121, no. Supplement 1, page 87, XP009144758 (2003). 43rd Annual Scientific Meeting of the British Society for Haematology, April 7-9, 2003.	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15/148839
	Filing Date	
	First Named Inventor	Leonard BELL
	Art Unit	N/A
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	AXJ-114BUSCN2

	38	Inoue et al., "Molecular genetics of paroxysmal nocturnal hemoglobinuria," Int J Hematol., Feb 77(2):107-112 (2003).	
	39	INT PNH INTEREST GRP: "Diagnosis management of paroxysmal nocturnal hemoglobinuria, Blood, vol. 106 (12):3699-3709, (2005) XP002482081	
	40	Jasinski, et al. "A novel mechanisms of complement-independent clearance of red cells deficient in glycosyl phosphatidylinositol-linked proteins" Blood, vol. 103(7):2827-2834 (2004)	
	41	Kaplan "Eculizumab Alexion," Current Opinion in Invest. Drugs, vol. 3(7),1017-1023 (2002)	
	42	Kato et al. "Deconstructing sickle cell disease: reappraisal of the role of hemolysis in the development of clinical subphenotypes," Blood Reviews, vol. 21: 37-47 (2007)	
	43	Kinoshita, Taroh, "Molecular pathogenesis of Paroxysmal Nocturnal Haemoglobinuria," XXVIth World Congress of the International Society of Haematology, Singapore, 25-29 August, 1996.	
	44	Lee et al., "Neisseria meningitidis Bacteremia in Association with Deficiency of the Sixth Component of Complement," Infection and Immunity, vol. 24(3): 656-660 (1979)	
	45	Luzzatto, et al., "Recent advances in biological and clinical aspects of paroxysmal nocturnal hemoglobinuria, Intl. J. Hematol. 84:104-112 (2006).	
	46	Macneil, "Eculizumab could be paroxysmal nocturnal hemoglobinuria breakthrough," MacNeil, J.S., 44th Annual Meeting of the American Society of Hematology, Medscape Medical News, (12/13/2002); [online]; Medscape from WebMD; [Retrieved on 2003-06-17. Retrieved from the world wide web at medscape.com/viewarticle/446336 XP0091 06338.	
	47	MERCK MANUAL of Diagnosis and Therapy (The), Seventeenth Edition, Chapter 127 Anemias, 849-883 (1999).	
	48	Hillmen et al., "Eculizumab, a C5 Complement-Blocking Antibody, Controls Hemolysis in Paroxysmal Nocturnal Hemoglobinuria (PNH) with Responses Maintained over prolonged Period of Therapy" Journal of The American Society of Hematology, Vol. 102(11), Abstract 1858 (2003).	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15/148839
	Filing Date	
	First Named Inventor	Leonard BELL
	Art Unit	N/A
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	AXJ-114BUSCN2

	49	Hillmen et al., "Eculizumab, a C5 complement-blocking antibody, controls haemolysis in paroxysmal nocturnal haemoglobinuria (PNH) with responses maintained over a prolonged period of therapy," British Journal of Haematology, Vol. 125 (Suppl. 1): 1-72, Abstract 5 (2004).	

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature	/JAMES L ROGERS/	Date Considered	08/08/2016

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15/148839
	Filing Date	
	First Named Inventor	Leonard BELL
	Art Unit	N/A
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	AXJ-114BUSCN2

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Jill Gorny Sloper, Esq./	Date (YYYY-MM-DD)	2016-05-06
Name/Print	Jill Gorny Sloper, Esq.	Registration Number	60760

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: May 12, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/148,839

Confirmation No.: 3159

Filed: May 6, 2016

Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT (SIDS)

Dear Sir:

Pursuant to 37 C.F.R. §§ 1.56, 1.97, and 1.98, the attention of the United States Patent and Trademark Office is hereby directed to the Office Actions listed herein. It is respectfully requested that the information be expressly considered during the prosecution of the above-identified application.

Applicants provide herewith copies of Office Actions from Applications previously made of record:

Office Actions (copies enclosed)				
Examiner's Initials	Serial No.:	Date Mailed from USPTO	Docket No.	Examiner
	15/260,888	04-11-2017	AXJ-114BUSCN3	J. Rogers
	15/284,015	04-11-2017	AXJ-114BUSCN4	J. Rogers

/JAMES L ROGERS/ 05/17/2017

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

The Examiner is requested to review the file histories of these applications, including cited references, Office Actions, Responses, etc., and is asked to contact Applicants' Attorney if the Examiner would like the Applicants to supply copies of any or all of the information included in any of these applications. For any of these applications, if Applicants' Attorney is not contacted by the Examiner with such a request, then it will be concluded that the Examiner has reviewed or will review the file content of these applications.

Applicants respectfully request that the Examiner initial the blank column next to the cited Office Actions, to indicate that the information has been considered by the Examiner. Alternatively, Applicants request that the Examiner insert the phrase, "All references considered except where lined through," on each page of the Information Disclosure Statement, along with the Examiner's initials.

The filing of this Information Disclosure Statement is not to be interpreted as a representation that the cited documents are material, that an exhaustive search has been conducted, or that no other relevant information exists. Nor shall the citation of any documents herein be construed *per se* as a representation that such document is prior art. Moreover, Applicants understand the Examiner will make an independent evaluation of the cited documents.

This Information Disclosure Statement is filed after the mailing date of a first Office Action on the merits, but before the mailing date of any of a Final Action under 37 C.F.R. § 1.113, a Notice of Allowance under 37 C.F.R. § 1.311 or an action that otherwise closes prosecution in this application (37 C.F.R. § 1.97(c)).

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

Samsung Bioepis Exhibit No. 1002
Page 1449

Please charge our Deposit Account No. 12-0080 in the amount of $180.00 covering the fee set forth in 37 C.F.R. § 1.17(p). The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN2.

Dated: May 12, 2017

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 217-4699 (Fax)
Attorney/Agent For Applicants

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (03-15)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	15/148839
Filing Date	
First Named Inventor	Leonard BELL
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	AXJ-114BUSCN2

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Patent citation information please click the Add button. Add

U.S.PATENT APPLICATION PUBLICATIONS Remove

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Published Application citation information please click the Add button. Add

FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1							

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

EFS Web 2.1.17

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15/148839
	Filing Date	
	First Named Inventor	Leonard BELL
	Art Unit	N/A
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	AXJ-114BUSCN2

	1	Mortazavi et al., "The spectrum of PIG-A gene mutations in a plastic anemia/paroxysmal nocturnal hemoglobinuria (AAIPNH): a high incidence of multiple mutations and evidence of a mutational hot spot," Blood 101 2833-2841 (2003).	
	2	Moyo et al., "Natural history of paroxysmal nocturnal haemoglobinuria using modern diagnostic assays," British J Haematology 126, 133-138 (2004).	
	3	Notice of Opposition for EP Patent No. 1720571, dated 3/18/2013.	
	4	Parker et al., Diagnosis and management of paroxysmal nocturnal hemoglobinuria, Blood, 106(12):3699-3709 (2005).	
	5	Paroxysmal nocturnal hemoglobinuria, included; PNH, included phosphatidylinositol glycan, class A, pseudogene 1, included; pigap1, included piga-related processed gene, included [online]. OM 1M-Online Medelian Inheritance in Man; John Hopkins University; [Retrieved on 2003-06-18]. Retrieved from the world wide web at ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=OMIM&dopt=detailed ...	
	6	Reiter et al., "Cell-free hemoglobin limits nitric oxide bioavailability in sickle-cell disease," Nature Medicine 8 (12):1383-1389 (2002).	
	7	Richards et al., "Evolution of GPI-Deficient Clones Predicts Clinical Course in Paroxysmal Nocturnal Haemoglobinuria," Blood, 104:Abstract 172 (2004).	
	8	Rodary et al., "Patient preference for either the EORTC QLQ-C30 or the FACIT Quality of Life (QOL) measures: a study performed in patient suffering from carcinoma of an unknown primary site (CUP)," European Journal of Cancer, Vol. 40, pp. 521-528 (2004).	
	9	Rosse et al., Immune-mediated hemolytic anemia, Hematology Am Soc Hematol Educ. Program, 48-62 (2004) XP002497825.	
	10	ROTHER et al., Eculizumab, a C5 complement-blocking antibody, abolishes hemolysis and reduces transfusion dependency in patients with paroxysmal nocturnal hemoglobinuria (PNH), Molecular Immunology, Vol. 40(2-4), page 197; XP009163498, (2003).	
	11	Rother et al., The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin, JAMA 293 (13):1653-1662 (2005).	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15/148839
	Filing Date	
	First Named Inventor	Leonard BELL
	Art Unit	N/A
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	AXJ-114BUSCN2

	12	Stebler et al., High-dose recombinant human erythropoietin for treatment of anemia in myelodysplastic syndromes and paroxysmal nocturnal hemoglobinuria: a pilot study, Exp. Hematol. 18:1204-1208 (1990).	
	13	Strauss et al. "Alternative pathway of complement in sickle cell disease," Pediat. Res., vol. 11:285-289 (1977)	
	14	Stuart et al., "Sickle-cell disease," Lancet, vol. 364:1343-60 (2004)	
	15	Tang and Pikal, Design of Freeze-Drying Processes for Pharmaceuticals: Practical Advice, Pharmaceutical Research, Vol. 21 (2):191-200 (2004).	
	16	Thomas et al., Inhibition of complement activity by humanized anti-C5 antibody and single chain Fv, Mol.Imm. 33 (17-18):1389-1401, XP002262113 (1996).	
	17	Vakeva, et al., "Myocardial infarction and apoptosis after myocardial ischemia and reperfusion," Circulation 97:2259-2267 (1998).	

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature	/JAMES L ROGERS/	Date Considered	08/08/2016

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15/148389
	Filing Date	
	First Named Inventor	Leonard BELL
	Art Unit	N/A
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	AXJ-114BUSCN2

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Jill Gorny Sloper, Esq./	Date (YYYY-MM-DD)	2016-05-06
Name/Print	Jill Gorny Sloper, Esq.	Registration Number	60760

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /J.L.R/

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15148839	BELL ET AL.
	Examiner	**Art Unit**
	JAMES ROGERS	1644

CPC							
Symbol						**Type**	**Version**
C07K		16	/	18		F	2013-01-01
A61K		31	/	7105		I	2013-01-01
A61K		2039	/	505		A	2013-01-01
A61K		38	/	00		A	2013-01-01
C07K		2317	/	24		A	2013-01-01
C07K		2317	/	565		A	2013-01-01
A61K		9	/	0019		I	2013-01-01
C07K		2317	/	51		A	2013-01-01
C07K		2317	/	515		A	2013-01-01
			/				
			/				
			/				
			/				
			/				
			/				

CPC Combination Sets								
Symbol					**Type**	**Set**	**Ranking**	**Version**
			/					
			/					

/JAMES ROGERS/ Examiner.Art Unit 1644		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	3	
/DANIEL E KOLKER/ Supervisory Patent Examiner.Art Unit 1644	05/26/2017	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	None

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15148839	BELL ET AL.
	Examiner	**Art Unit**
	JAMES ROGERS	1644

US ORIGINAL CLASSIFICATION		INTERNATIONAL CLASSIFICATION										
CLASS	SUBCLASS	CLAIMED					NON-CLAIMED					
		A	6	1	K	39 / 00 (2006.01.01)						
CROSS REFERENCE(S)		A	6	1	K	9 / 00 (2006.0)						
		C	0	7	K	16 / 18 (2006.0)						
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)	C	0	7	K	19 / 00 (2006.0)						

/JAMES ROGERS/ Examiner.Art Unit 1644 (Assistant Examiner)	(Date)	**Total Claims Allowed:** 3	
/DANIEL E KOLKER/ Supervisory Patent Examiner.Art Unit 1644 (Primary Examiner)	05/26/2017 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure None

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15148839	BELL ET AL.
	Examiner	**Art Unit**
	JAMES ROGERS	1644

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☒ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original

/JAMES ROGERS/ Examiner.Art Unit 1644 (Assistant Examiner)	(Date)	**Total Claims Allowed:** 3	
/DANIEL E KOLKER/ Supervisory Patent Examiner.Art Unit 1644 (Primary Examiner)	05/26/2017 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure None

EAST Search History

EAST Search History (Prior Art)

<This search history is empty>

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	50	(("300" near2 mg) near10 (eculizumab or soliris))	US-PGPUB; USPAT	OR	ON	2017/05/19 12:32
L2	2	(("300" near2 mg) near10 (eculizumab or soliris)).clm.	US-PGPUB; USPAT	OR	ON	2017/05/19 12:32

5/19/2017 12:32:35 PM

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15148839	BELL ET AL.
	Examiner	Art Unit
	JAMES ROGERS	1644

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
STN: DISSABS MEETINGS BIOSCI CAPLUS MEDICINE	8/8/2016	JR
EAST: PGPUB, USPAT, JPO, EPO, INVENTOR SEARCH	8/8/2016	JR
updated	3/2/2017	JR

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
	USPAT, USPAd	5/19/2017	JR

	/James Rogers/ Examiner Art Unit 1644

To: chris.schlauch@nelsonmullins.com,ipqualityassuranceboston@nelsonmullins.com,ipboston.docketing@nel
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 133485

Jun 07, 2017 04:30:36 AM

Dear PAIR Customer:

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 133485 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:
The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
15148839	NOA	06/07/2017	AXJ-114BUSCN2
	1449	06/07/2017	AXJ-114BUSCN2
	1449	06/07/2017	AXJ-114BUSCN2
	1449	06/07/2017	AXJ-114BUSCN2
	1449	06/07/2017	AXJ-114BUSCN2

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

PART B–FEE(S) TRANSMITTAL

Complete and send this form, together with the applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22314-1450
or Fax **(571)-273-2885**

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note Use Block 1 for any change of address)

133485 7590 06/07/2017
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's Name)
(S-signature)
(Date)

APPLICATION NO	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO	CONFIRMATION NO
15/148,839	05/06/2016	Leonard BELL	AXJ-114BUSCN2	3159

TITLE OF INVENTION: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

APPLN: TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960.00	$0	$0	$963.00	09/07/2017

EXAMINER	ART UNIT	CLASS-SUBCLASS
J. L. Rogers	1644	424-133100

1. Change of correspondence address or indication of "Fee Address" (37CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☒ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered attorney or agent. If no name is listed, no name will be printed

1. Tara Rahemba, Esq.
2. Jane E. Remillard, Esq.
3. Jill Gorny Sloper, Esq.

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

1a. NAME OF ASSIGNEE: Alexion Pharmaceuticals, Inc.

(B) RESIDENCE: (CITY and STATE OR COUNTRY): New Haven, Connecticut

Please check the appropriate assignee categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:

☒ Issue Fee

☐ Publication Fee (No small entity discount permitted)

☒ Advance Order - # of Copies 1

4b. Payment of Fees(s): **(Please first reapply and previously paid fee shown above)**

☐ A check is enclosed

☐ Payment be credit card. Form PTO-2038 is attached

☒ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number 12-0080 (enclose an extra copy of this form)

5. Change of Entity Status (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29
NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

☐ Applicant asserting small entity status. See 37 CFR 1.27
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

☐ Applicant changing to regnlar undiscounted fee status.
NOTE: Checking this box will be taken to be notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Jill Gorny Sloper, Esq./ Date June 9, 2017

Typed or printed name Jill Gorny Sloper, Esq. Registration No. 60,760

Samsung Bioepis Exhibit No. 1002
Page 1462

Electronic Patent Application Fee Transmittal

Application Number:	15148839
Filing Date:	06-May-2016
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Attorney Docket Number:	AXJ-114BUSCN2

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
UTILITY APPL ISSUE FEE	1501	1	960	960
PUBL. FEE- EARLY, VOLUNTARY, OR NORMAL	1504	1	0	0
Extension-of-Time:				
Miscellaneous:				
Printed copy of patent - no color	8001	1	3	3
		Total in USD ($)		**963**

Electronic Acknowledgement Receipt	
EFS ID:	29446630
Application Number:	15148839
International Application Number:	
Confirmation Number:	3159
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Customer Number:	133485
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Filer Authorized By:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN2
Receipt Date:	09-JUN-2017
Filing Date:	06-MAY-2016
Time Stamp:	13:18:59
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$963
RAM confirmation Number	060917INTEFSW00010524120080
Deposit Account	120080
Authorized User	Jill Sloper
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.16 (National application filing, search, and examination fees) 37 CFR 1.17 (Patent application and reexamination processing fees)	

37 CFR 1.19 (Document supply fees)

37 CFR 1.20 (Post Issuance fees)

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Change of Address	AXJ_114BUSCN2_Change_Fee.pdf	122496 7d65ab8d57c8e420e78168422354 2ea18cf1df1f	no	1
Warnings:					
Information:					
2	Issue Fee Payment (PTO-85B)	AXJ_114BUSCN2_Issue_Fee_Transmittal.pdf	119826 b08d5230837863a055c092c29df5a87faaafa45d	no	1
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	33946 6c2653b7c9c05dd3552aff0ddb9eca580f73971f	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	276268		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/47 (03-09)
Approved for use through 05/31/2015. OMB 0651-0016
U.S. Patent and Trademark Office; U. S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

"FEE ADDRESS" INDICATION FORM

Address to:
Mail Stop M Correspondence
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

- OR -

Fax to:
571-273-6500

INSTRUCTIONS: The issue fee must have been paid for application(s) listed on this form. In addition, only an address represented by a Customer Number can be established as the fee address for maintenance fee purposes (hereafter, fee address). A fee address should be established when correspondence related to maintenance fees should be mailed to a different address than the correspondence address for the application. **When to check the first box below:** If you have a Customer Number to represent the fee address. **When to check the second box below:** If you have no Customer Number representing the desired fee address, in which case a completed Request for Customer Number (PTO/SB/125) must be attached to this form. For more information on Customer Numbers, see the Manual of Patent Examining Procedure (MPEP) § 403.

For the following listed application(s), please recognize as the "Fee Address" under the provisions of 37 CFR 1.363 the address associated with:

[X] Customer Number: 127532

OR

[ ] The attached Request for Customer Number (PTO/SB/125) form.

PATENT NUMBER (if known)	APPLICATION NUMBER
	15/148,839

Completed by (check one):

[ ] Applicant/Inventor

[X] Attorney or Agent of record 60,760 (Reg. No.)

[ ] Assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)

[ ] Assignee recorded at Reel _____ Frame _____

/Jill Gorny Sloper, Esq./
Signature

Jill Gorny Sloper, Esq.
Typed or printed name

(617) 217-4630
Requester's telephone number

June 9, 2017
Date

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more that one signature is required, see below*.

[ ] * Total of 1 forms are submitted.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: June 9, 2017 Signature: /Jill Gorny Sloper, Esq./ (Jill Gorny Sloper, Esq.)

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/148,839	08/01/2017	9718880	AXJ-114BUSCN2	3159

133485 7590 07/12/2017

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

Leonard BELL, Woodbridge, CT;
Alexion Pharmaceuticals, Inc., New Haven, CT;
Russell P. ROTHER, Oklahoma City, OK;
Mark J. EVANS, Radnor, PA;

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

To: chris.schlauch@nelsonmullins.com,ipqualityassuranceboston@nelsonmullins.com,ipboston.docketing@nel
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 133485

Jul 13, 2017 08:50:28 AM

Dear PAIR Customer:

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 133485 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:
The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
15148839	ISSUE.NTF	07/12/2017	AXJ-114BUSCN2

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

PTO/SB/44 (09-07)
Approved for use through 01/31/2020. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.
(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.	:	9718880 B2
APPLICATION NO.	:	15/148,839
ISSUE DATE	:	August 1, 2017
INVENTOR(S)	:	Leonard BELL *et al.*

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On page 2, in column 1, under “Other Publications”, line 6, delete “Nocturanl” and insert - - Nocturnal - -, therefor.

On page 2, in column 1, under “Other Publications”, line 8, delete “identifer” and insert - - identifier - -, therefor.

On page 2, in column 1, under “Other Publications”, line 15, delete “Rather” and insert - - Rother - -, therefor.

On page 2, in column 1, under “Other Publications”, line 19, delete “"Solids” and insert - - "Soliris - -, therefor.

On page 2, in column 2, under “Other Publications”, line 17, delete “EMAIHMPC” and insert - - EMA/HMPC - -, therefor.

On page 2, in column 2, under “Other Publications”, line 38, delete “Dsborne,” and insert - - Osborne, - -, therefor.

On page 2, in column 2, under “Other Publications”, line 40, delete “Dsbourn,” and insert - - Osbourn, - -, therefor.

On page 3, in column 1, under “Other Publications”, line 1, delete “2007,” and insert - - 2006, - -, therefor.

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):
Jill Gorny Sloper, Esq.
NELSON MULLINS RILEY & SCARBOROUGH LLP 1
One Post Office Square
Boston, Massachusetts 02109-2127

PTO/SB/44 (09-07)
Approved for use through 01/31/2020. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.
(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.	:	9718880 B2
APPLICATION NO.	:	15/148,839
ISSUE DATE	:	August 1, 2017
INVENTOR(S)	:	Leonard BELL *et al.*

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On page 3, in column 1, under “Other Publications”, lines 18-19, delete “mi mOYUG/is 1 14/ai 17208612” and insert - - mimOYUG/is114/ai17208612 - -, therefor.

On page 3, in column 1, under “Other Publications”, line 23, delete “sub press.cfm” and insert - - subpress.cfm - -, therefor.

On page 3, in column 1, under “Other Publications”, line 35, delete “Insrum.” and insert - - Instrum. - -, therefor.

On page 3, in column 1, under “Other Publications”, line 42, delete “Neisseia” and insert - - Neisseria - -, therefor.

On page 3, in column 1, under “Other Publications”, line 53, delete “sub press.” and insert - - subpress. - -, therefor.

On page 3, in column 1, under “Other Publications”, line 56, delete “Hemoglobinuira” and insert - - Hemoglobinuria - -, therefor.

On page 3, in column 1, under “Other Publications”, line 59, delete “view newsitem.aspx” and insert - - viewnewsitem.aspx - -, therefor.

On page 3, in column 1, under “Other Publications”, line 60, delete “Phamaceuticals:” and insert - - Pharmaceuticals: - -, therefor.

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):
Jill Gorny Sloper, Esq.
NELSON MULLINS RILEY & SCARBOROUGH LLP 2
One Post Office Square
Boston, Massachusetts 02109-2127

PTO/SB/44 (09-07)
Approved for use through 01/31/2020. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.
(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.	:	9718880 B2
APPLICATION NO.	:	15/148,839
ISSUE DATE	:	August 1, 2017
INVENTOR(S)	:	Leonard BELL *et al.*

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On page 3, in column 1, under “Other Publications”, line 64, delete “Pharmaceuitcals” and insert - - Pharmaceuticals - -, therefor.

On page 3, in column 2, under “Other Publications”, line 2, delete “Domponent,"” and insert - - component," - -, therefor.

On page 3, in column 2, under “Other Publications”, line 17, delete “hourse;” and insert - - horse; - -, therefor.

On page 3, in column 2, under “Other Publications”, line 40, delete “membranetargeted” and insert - - membrane targeted - -, therefor.

On page 3, in column 2, under “Other Publications”, line 41, delete “107(5)2131-2137” and insert - - 107(5):2131-2137 - -, therefor.

On page 3, in column 2, under “Other Publications”, line 53, delete “hemglobinuria,"” and insert - - hemoglobinuria," - -, therefor.

On page 3, in column 2, under “Other Publications”, line 53, delete “Hermal.” and insert - - Hematol. - -, therefor.

On page 3, in column 2, under “Other Publications”, line 64, delete “17:125-132” and insert - - 77:125-132 - -, therefor.

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):
Jill Gorny Sloper, Esq.
NELSON MULLINS RILEY & SCARBOROUGH LLP 3
One Post Office Square
Boston, Massachusetts 02109-2127

PTO/SB/44 (09-07)
Approved for use through 01/31/2020. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.
(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.	:	9718880 B2
APPLICATION NO.	:	15/148,839
ISSUE DATE	:	August 1, 2017
INVENTOR(S)	:	Leonard BELL *et al.*

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On page 3, in column 2, under “Other Publications”, line 70, delete “iocturnal” and insert - - nocturnal - -, therefor.

On page 4, in column 2, under “Other Publications”, line 6, delete “modem” and insert - - modern - -, therefor.

On page 4, in column 2, under “Other Publications”, line 15, delete “Medelian” and insert - - Mendelian - -, therefor.

In column 1, line 23, delete “Seq _Listing.txt” and insert - - Seq_Listing.txt - -, therefor.

In column 1, lines 45-46, delete “heomoglobinuria” and insert - - hemoglobinuria - -, therefor.

In column 1, line 66, delete “II” and insert - - III - -, therefor.

In column 4, line 2, delete “antibodies” and insert - - antibodies, - -, therefor.

In column 4, line 43, delete “NO:5,” and insert - - NO: 5, - -, therefor.

In column 4, line 43, delete “NO:6,” and insert - - NO: 6, - -, therefor.

In column 4, line 44, delete “NO:7,” and insert - - NO: 7, - -, therefor.

In column 4, line 47, delete “NO:8,” and insert - - NO: 8, - -, therefor.

In column 4, line 47, delete “NO:9,” and insert - - NO: 9, - -, therefor.

In column 4, line 47, delete “NO:10.” and insert - - NO: 10. - -, therefor.

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):
Jill Gorny Sloper, Esq.
NELSON MULLINS RILEY & SCARBOROUGH LLP 4
One Post Office Square
Boston, Massachusetts 02109-2127

PTO/SB/44 (09-07)
Approved for use through 01/31/2020. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.
(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.	:	9718880 B2
APPLICATION NO.	:	15/148,839
ISSUE DATE	:	August 1, 2017
INVENTOR(S)	:	Leonard BELL *et al.*

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In column 5, line 45, delete “Instrument” and insert - - Instrument. - -, therefor.

In column 6, line 33, delete “README.htm-1)” and insert - - README.html) - -, therefor.

In column 7, line 49, delete “Neisseia” and insert - - Neisseria - -, therefor.

In column 7, line 57, delete “scrum” and insert - - serum - -, therefor.

In column 7, line 57, delete “T,” and insert - - I, - -, therefor.

In column 8, line 48, delete “at” and insert - - et - -, therefor.

In column 8, line 53, delete “Measurer” and insert - - Measures - -, therefor.

In column 9, line 51, delete “if” and insert - - life - -, therefor.

In column 10, line 28, delete “(RNAI)” and insert - - (RNAi) - -, therefor.

In column 10, line 54, delete “Antibodies” and insert - - antibodies - -, therefor.

In column 11, line 32, delete “2d” and insert - - 2nd - -, therefor.

In column 12, line 8, delete “anaphylatoxic” and insert - - anaphylatoxin - -, therefor.

In column 12, line 22, delete “at” and insert - - et - -, therefor.

In column 12, line 30, delete “at” and insert - - et - -, therefor.

In column 12, line 41, delete “immunopharmaccticals” and insert - - immunopharmaceuticals - -, therefor.

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):
Jill Gorny Sloper, Esq.
NELSON MULLINS RILEY & SCARBOROUGH LLP 5
One Post Office Square
Boston, Massachusetts 02109-2127

PTO/SB/44 (09-07)
Approved for use through 01/31/2020. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.
(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.	:	9718880 B2
APPLICATION NO.	:	15/148,839
ISSUE DATE	:	August 1, 2017
INVENTOR(S)	:	Leonard BELL *et al.*

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In column 13, line 15, delete “F(ab')2” and insert - - $F(ab')_2$ - -, therefor.

In column 13, line 45, delete “at” and insert - - et - -, therefor.

In column 13, line 56, delete “at” and insert - - et - -, therefor.

In column 14, line 15, delete “(see.” and insert - - (see, - -, therefor.

In column 14, line 29, delete “antibodies”” and insert - - antibodies - -, therefor.

In column 15, line 1, delete “prepared,” and insert - - prepared - -, therefor.

In column 15, line 5, after “6,331,415;” insert - - U.S. Pat. No. - -.

In column 15, line 10, delete “at” and insert - - et - -, therefor.

In column 15, line 17, delete “fill-length” and insert - - full-length - -, therefor.

In column 15, line 38, delete “at” and insert - - et - -, therefor.

In column 15, line 39, delete “at” and insert - - et - -, therefor.

In column 15, line 40, delete “at” and insert - - et - -, therefor.

In column 15, line 42, delete “Method” and insert - - Methods - -, therefor.

In column 16, line 6, delete “pet” and insert - - per - -, therefor.

In column 18, line 41, delete “aid” and insert - - and - -, therefor.

In column 18, line 59, after “(mg/mL)]” insert - - . - -.

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):
Jill Gorny Sloper, Esq.
NELSON MULLINS RILEY & SCARBOROUGH LLP 6
One Post Office Square
Boston, Massachusetts 02109-2127

PTO/SB/44 (09-07)
Approved for use through 01/31/2020. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.
(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.	:	9718880 B2
APPLICATION NO.	:	15/148,839
ISSUE DATE	:	August 1, 2017
INVENTOR(S)	:	Leonard BELL *et al.*

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In column 19, line 31, delete “farther” and insert - - further - -, therefor.

In column 19, line 35, delete “needle-lees” and insert - - needle-less - -, therefor.

In column 20, line 2, delete “meningoooccal” and insert - - meningococcal - -, therefor.

In column 20, line 8, delete “prior-12-month” and insert - - prior 12-month - -, therefor.

In column 20, line 14, delete “PBRC” and insert - - PRBC - -, therefor.

In column 20, line 25, delete “level≥11.5” and insert - - level≥1.5 - -, therefor.

In column 21, line 24, delete “HI” and insert - - III - -, therefor.

In column 21, line 63, delete “Pharmokinetics” and insert - - Pharmacokinetics - -, therefor.

In column 22, line 18, delete “2418.9*140.3” and insert - - 2418.9±140.3 - -, therefor.

In column 22, line 50, delete “28.1±12.0%” and insert - - 28.1±2.0% - -, therefor.

In column 27, lines 41-42, delete “functioning” and insert - - functioning, - -, therefor.

In column 28, line 3, delete “8” and insert - - a - -, therefor.

In column 28, line 21, after “independent” insert - - . - -.

In column 28, line 60, delete “Bemshaw” and insert - - Bernshaw - -, therefor.

In column 29, line 24, delete “Celia” and insert - - Cella - -, therefor.

In column 29, line 52, after “Abstract” insert - - . - -.

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):
Jill Gorny Sloper, Esq.
NELSON MULLINS RILEY & SCARBOROUGH LLP 7
One Post Office Square
Boston, Massachusetts 02109-2127

PTO/SB/44 (09-07)
Approved for use through 01/31/2020. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.
(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.	:	9718880 B2
APPLICATION NO.	:	15/148,839
ISSUE DATE	:	August 1, 2017
INVENTOR(S)	:	Leonard BELL *et al.*

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In column 30, line 2, delete “Patients.” and insert - - Patients - -, therefor.

In column 30, line 25, delete

“PKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH**AN**KTKPRE” and insert - -

PKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH**NA**KTKPRE - -, therefor.

In column 30, line 27, delete

“P**EPE**QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY and insert - -

P**REP**QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY - -, therefor.

In column 30, line 34, delete

“MDMRVPAQLLGLLLL**SLR**GARCDIQMTQSPSSLSASVGDRVTITCGASE” and insert - -

MDMRVPAQLLGLLLL**WLR**GARCDIQMTQSPSSLSASVGDRVTITCGASE - -, therefor.

In column 30, line 61, delete “Eculizmuab” and insert - - Eculizumab - -, therefor.

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):
Jill Gorny Sloper, Esq.
NELSON MULLINS RILEY & SCARBOROUGH LLP 8
One Post Office Square
Boston, Massachusetts 02109-2127

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: September 22, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of:
Leonard Bell *et al.*

Patent No.: 9718880 B2

Issued: August 1, 2017

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Attention: Certificate of Correction Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
PURSUANT TO 37 C.F.R. § 1.323

Dear Sir:

Upon reviewing the above-identified patent, Patentee noted typographical errors which should be corrected.

On page 2, in column 1, under "Other Publications", line 6, delete "Nocturanl" and insert - - Nocturnal - -, therefor.

On page 2, in column 1, under "Other Publications", line 8, delete "identifer" and insert - - identifier - -, therefor.

On page 2, in column 1, under "Other Publications", line 15, delete "Rather" and insert - - Rother - -, therefor.

On page 2, in column 1, under “Other Publications”, line 19, delete “"Solids” and insert - - "Soliris - -, therefor.

On page 2, in column 2, under “Other Publications”, line 17, delete “EMAIHMPC” and insert - - EMA/HMPC - -, therefor.

On page 2, in column 2, under “Other Publications”, line 38, delete “Dsborne,” and insert - - Osborne, - -, therefor.

On page 2, in column 2, under “Other Publications”, line 40, delete “Dsbourn,” and insert - - Osbourn, - -, therefor.

On page 3, in column 1, under “Other Publications”, line 1, delete “2007,” and insert - - 2006, - -, therefor.

On page 3, in column 1, under “Other Publications”, lines 18-19, delete “mi mOYUG/is 1 14/ai 17208612” and insert - - mimOYUG/is114/ai17208612 - -, therefor.

On page 3, in column 1, under “Other Publications”, line 23, delete “sub press.cfm” and insert - - subpress.cfm - -, therefor.

On page 3, in column 1, under “Other Publications”, line 35, delete “Insrum.” and insert - - Instrum. - -, therefor.

On page 3, in column 1, under “Other Publications”, line 42, delete “Neisseia” and insert - - Neisseria - -, therefor.

On page 3, in column 1, under “Other Publications”, line 53, delete “sub press.” and insert - - subpress. - -, therefor.

On page 3, in column 1, under “Other Publications”, line 56, delete “Hemoglobinuira” and insert - - Hemoglobinuria - -, therefor.

On page 3, in column 1, under “Other Publications”, line 59, delete “view newsitem.aspx” and insert - - viewnewsitem.aspx - -, therefor.

On page 3, in column 1, under “Other Publications”, line 60, delete “Phamaceuticals:” and insert - - Pharmaceuticals: - -, therefor.

On page 3, in column 1, under “Other Publications”, line 64, delete “Pharmaceuitcals” and insert - - Pharmaceuticals - -, therefor.

On page 3, in column 2, under “Other Publications”, line 2, delete “Domponent,"” and insert - - component," - -, therefor.

On page 3, in column 2, under “Other Publications”, line 17, delete “hourse;” and insert - - horse; - -, therefor.

On page 3, in column 2, under “Other Publications”, line 40, delete “membranetargeted” and insert - - membrane targeted - -, therefor.

On page 3, in column 2, under “Other Publications”, line 41, delete “107(5)2131-2137” and insert - -107(5):2131-2137 - -, therefor.

On page 3, in column 2, under “Other Publications”, line 53, delete “hemglobinuria,"” and insert - - hemoglobinuria," - -, therefor.

On page 3, in column 2, under “Other Publications”, line 53, delete “Hermal.” and insert - - Hematol. - -, therefor.

On page 3, in column 2, under “Other Publications”, line 64, delete “17:125-132” and insert - - 77:125-132 - -, therefor.

On page 3, in column 2, under “Other Publications”, line 70, delete “iocturnal” and insert - - nocturnal - -, therefor.

On page 4, in column 2, under “Other Publications”, line 6, delete “modem” and insert - - modern - -, therefor.

On page 4, in column 2, under “Other Publications”, line 15, delete “Medelian” and insert - - Mendelian - -, therefor.

In the Specification:

In column 1, line 23, delete “Seq _Listing.txt” and insert - - Seq_Listing.txt - -, therefor.

In column 1, lines 45-46, delete “heomoglobinuria” and insert - - hemoglobinuria - -, therefor.

In column 1, line 66, delete “II” and insert - - III - -, therefor.

In column 4, line 2, delete “antibodies” and insert - - antibodies, - -, therefor.

In column 4, line 43, delete “NO:5,” and insert - - NO: 5, - -, therefor.

In column 4, line 43, delete “NO:6,” and insert - - NO: 6, - -, therefor.

In column 4, line 44, delete “NO:7,” and insert - - NO: 7, - -, therefor.

In column 4, line 47, delete “NO:8,” and insert - - NO: 8, - -, therefor.

In column 4, line 47, delete “NO:9,” and insert - - NO: 9, - -, therefor.

In column 4, line 47, delete “NO:10.” and insert - - NO: 10. - -, therefor.

In column 5, line 45, delete “Instrument” and insert - - Instrument. - -, therefor.

In column 6, line 33, delete “READMB.htm-1)” and insert - - README.html) - -, therefor.

In column 7, line 49, delete “Neisseia” and insert - - Neisseria - -, therefor.

In column 7, line 57, delete “scrum” and insert - - serum - -, therefor.

In column 7, line 57, delete “T,” and insert - - I, - -, therefor.

In column 8, line 48, delete “at” and insert - - et - -, therefor.

In column 8, line 53, delete “Measurer” and insert - - Measures - -, therefor.

In column 9, line 51, delete “if” and insert - - life - -, therefor.

In column 10, line 28, delete “(RNAI)” and insert - - (RNAi) - -, therefor.

In column 10, line 54, delete “Antibodies” and insert - - antibodies - -, therefor.

In column 11, line 32, delete “2d” and insert - - 2nd - -, therefor.

In column 12, line 8, delete “anaphylatoxic” and insert - - anaphylatoxin - -, therefor.

In column 12, line 22, delete “at” and insert - - et - -, therefor.

In column 12, line 30, delete “at” and insert - - et - -, therefor.

In column 12, line 41, delete “immunopharmaccticals” and insert - - immunopharmaceuticals - -, therefor.

In column 13, line 15, delete “F(ab')2” and insert - - $F(ab')_2$ - -, therefor.

In column 13, line 45, delete “at” and insert - - et - -, therefor.

In column 13, line 56, delete “at” and insert - - et - -, therefor.

In column 14, line 15, delete “(see.” and insert - - (see, - -, therefor.

In column 14, line 29, delete “antibodies”” and insert - - antibodies - -, therefor.

In column 15, line 1, delete “prepared,” and insert - - prepared - -, therefor.

In column 15, line 5, after “6,331,415;” insert - - U.S. Pat. No. - -.

In column 15, line 10, delete “at” and insert - - et - -, therefor.

In column 15, line 17, delete “fill-length” and insert - - full-length - -, therefor.

In column 15, line 38, delete “at” and insert - - et - -, therefor.

In column 15, line 39, delete “at” and insert - - et - -, therefor.

In column 15, line 40, delete “at” and insert - - et - -, therefor.

In column 15, line 42, delete “Method” and insert - - Methods - -, therefor.

In column 16, line 6, delete “pet” and insert - - per - -, therefor.

In column 18, line 41, delete “aid” and insert - - and - -, therefor.

In column 18, line 59, after “(mg/mL)]” insert - - . - -.

In column 19, line 31, delete “farther” and insert - - further - -, therefor.

In column 19, line 35, delete “needle-lees” and insert - - needle-less - -, therefor.

In column 20, line 2, delete “meningoooccal” and insert - - meningococcal - -, therefor.

In column 20, line 8, delete “prior-12-month” and insert - - prior 12-month - -, therefor.

In column 20, line 14, delete “PBRC” and insert - - PRBC - -, therefor.

In column 20, line 25, delete “level≥11.5” and insert - - level≥1.5 - -, therefor.

In column 21, line 24, delete “HI” and insert - - III - -, therefor.

In column 21, line 63, delete “Pharmokinetics” and insert - - Pharmacokinetics - -, therefor.

In column 22, line 18, delete “2418.9*140.3” and insert - - 2418.9±140.3 - -, therefor.

In column 22, line 50, delete “28.1±12.0%” and insert - - 28.1±2.0% - -, therefor.

In column 27, lines 41-42, delete “functioning” and insert - - functioning, - -, therefor.

In column 28, line 3, delete “8” and insert - - a - -, therefor.

In column 28, line 21, after “independent” insert - - . - -.

In column 28, line 60, delete “Bemshaw” and insert - - Bernshaw - -, therefor.

In column 29, line 24, delete “Celia” and insert - - Cella - -, therefor.

In column 29, line 52, after “Abstract” insert - - . - -.

In column 30, line 2, delete “Patients.” and insert - - Patients - -, therefor.

In column 30, line 25, delete

“PKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH**A**N**K**TKPRE”

and insert

- - PKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH**NA**KTKPRE - -,

therefor.

In column 30, line 27, delete

“P**EPE**QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY

and insert

- - P**REP**QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY - -, therefor.

In column 30, line 34, delete

"MDMRVPAQLLGLLLL**SLR**GARCDIQMTQSPSSLSASVGDRVTITCGASE"

and insert

- - MDMRVPAQLLGLLLL**WLR**GARCDIQMTQSPSSLSASVGDRVTITCGASE - -, therefor.

In column 30, line 61, delete "Eculizmuab" and insert - - Eculizumab - -, therefor.

The errors now sought to be corrected are typographical errors the correction of which does not involve new matter or require reexamination.

Transmitted herewith is a proposed Certificate of Correction effecting such amendment. Patentee respectfully solicits the granting of the requested Certificate of Correction.

Please charge our Deposit Account No. 12-0080 in the amount of $100.00 covering the fee set forth in 37 C.F.R. § 1.20(a). The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN2.

Dated: September 22, 2017

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 217-4699 (Fax)
Attorney/Agent For Applicant

Electronic Patent Application Fee Transmittal

Application Number:	15148839
Filing Date:	06-May-2016
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Attorney Docket Number:	AXJ-114BUSCN2

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Certificate of correction	1811	1	100	100

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**100**

Electronic Acknowledgement Receipt	
EFS ID:	30438084
Application Number:	15148839
International Application Number:	
Confirmation Number:	3159
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Customer Number:	133485
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Filer Authorized By:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN2
Receipt Date:	22-SEP-2017
Filing Date:	06-MAY-2016
Time Stamp:	13:42:03
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$100
RAM confirmation Number	092217INTEFSW00010824120080
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Certificate of Correction	AXJ_114BUSCN2_Proposed_CoC.pdf	170245 599eefd8e4319c0183286818d06b4ced5a27a2ad	no	8
Warnings:					
Information:					
2	Request for Certificate of Correction	AXJ_114BUSCN2_Request_CoC.pdf	221826 97555056fe49967622b096993b74043e55b03a06	no	6
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30609 85f7d64ff350f7a2f0a3fa280bd6196bc46260c3	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	422680		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/148,839	05/06/2016	Leonard BELL	AXJ-114BUSCN2	3159

133485 7590 10/06/2017

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
10/06/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

Notice of Pre-AIA or AIA Status

The present application is being examined under the pre-AIA first to invent provisions.

The proposed changes are typographical errors. The changes to the sequences which appear in column 30 are obvious errors with obvious corrections.

The change in column 30 line 25 finds support in SEQ ID NO:2 of the sequence listing; residues 286 – 289 are His Asn Ala Lys respectively.

The change in column 30 line 27 finds support in SEQ ID NO:2 of the sequence listing; residues 344 – 347 are Pro Arg Glu Pro respectively.

The change in column 30 line 34 finds support in SEQ ID NO:3 of the sequence listing; residues 16-18 are Trp Leu Arg respectively.

/DANIEL E KOLKER/
Supervisory Patent Examiner, Art Unit 1644

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Patent No.: 9718880
Issue Date: 01 August 2017
Appl. No.: 15/148,839
Filed: 06 May 2016

PART (A) RESPONSE FOR CERTIFICATES OF CORRECTION

This is a decision on the Certificate of Correction request filed 22 September 2017.

The request for issuance of Certificate of Correction for the above-identified correction(s) under the provisions of 37 CFR 1.322 and/or 1.323 is hereby:

(Check one)
☑ Approved ☐ Approved in Part ☐ Denied

Comments: See attached

PART (B) PETITION UNDER 37 CFR 1.324 OR 37 CFR 1.48

☐ This is a decision on the petition filed ____ to correct inventorship under 37 CFR 1.324.

☐ This is a decision on the request under 37 CFR 1.48, petition filed ____. In view of the fact that the patent has already issued, the request under 37 CFR 1.48 has been treated as a petition to correct inventorship under 37 CFR 1.324.

The petition is hereby: ☐ Granted ☐ Dismissed

Comment: ____

The patented filed is being forwarded to Certificate of Corrections Branch for issuance of a certificate naming only the actual inventor or inventors.

/DANIEL E KOLKER/
Supervisory Patent Examiner, Art Unit 1644
Technology Center 1600
Phone: (571)272-3181

Certificates of Correction Branch email: CustomerServiceCoC@uspto.gov CoC Central Phone Number: (703) 756-1814

To: chris.schlauch@nelsonmullins.com,ipqualityassuranceboston@nelsonmullins.com,ipboston.docketing@nel
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 133485

Oct 07, 2017 07:45:51 AM

Dear PAIR Customer:

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 133485 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:
The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
15148839	COCX	10/06/2017	AXJ-114BUSCN2

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 9,718,880 B2
APPLICATION NO. : 15/148839
DATED : August 1, 2017
INVENTOR(S) : Leonard Bell et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title Page

Item (56)

On page 2, in Column 1, under "Other Publications", Line 6, delete "Nocturanl" and insert -- Nocturnal --, therefor.

On page 2, in Column 1, under "Other Publications", Line 8, delete "identifer" and insert -- identifier --, therefor.

On page 2, in Column 1, under "Other Publications", Line 15, delete "Rather" and insert -- Rother --, therefor.

On page 2, in Column 1, under "Other Publications", Line 19, delete ""Solids" and insert -- "Soliris --, therefor.

On page 2, in Column 2, under "Other Publications", Line 17, delete "EMAIHMPC" and insert -- EMA/HMPC --, therefor.

On page 2, in Column 2, under "Other Publications", Line 38, delete "Dsborne," and insert -- Osborne, --, therefor.

On page 2, in Column 2, under "Other Publications", Line 40, delete "Dsbourn," and insert -- Osbourn, --, therefor.

On page 3, in Column 1, under "Other Publications", Line 1, delete "2007," and insert -- 2006, --, therefor.

Signed and Sealed this
Twenty-fourth Day of October, 2017

Joseph Matal

Joseph Matal
Performing the Functions and Duties of the
Under Secretary of Commerce for Intellectual Property and
Director of the United States Patent and Trademark Office

On page 3, in Column 1, under "Other Publications", Lines 18-19, delete "mi mOYUG/is 1 14/ai 17208612" and insert -- mimOYUG/is114/ai17208612 --, therefor.

On page 3, in Column 1, under "Other Publications", Line 23, delete "sub press.cfm" and insert -- subpress.cfm --, therefor.

On page 3, in Column 1, under "Other Publications", Line 35, delete "Insrum." and insert -- Instrum. --, therefor.

On page 3, in Column 1, under "Other Publications", Line 42, delete "Neisseia" and insert -- Neisseria --, therefor.

On page 3, in Column 1, under "Other Publications", Line 53, delete "sub press." and insert -- subpress. --, therefor.

On page 3, in Column 1, under "Other Publications", Line 56, delete "Hemoglobinuira" and insert -- Hemoglobinuria --, therefor.

On page 3, in Column 1, under "Other Publications", Line 59, delete "view newsitem.aspx" and insert -- viewnewsitem.aspx --, therefor.

On page 3, in Column 1, under "Other Publications", Line 60, delete "Phamaceuticals:" and insert -- Pharmaceuticals: --, therefor.

On page 3, in Column 1, under "Other Publications", Line 64, delete "Pharmaceuitcals" and insert -- Pharmaceuticals --, therefor.

On page 3, in Column 2, under "Other Publications", Line 2, delete "Domponent,"" and insert -- component," --, therefor.

On page 3, in Column 2, under "Other Publications", Line 17, delete "hourse;" and insert -- horse; --, therefor.

On page 3, in Column 2, under "Other Publications", Line 40, delete "membranetargeted" and insert -- membrane targeted --, therefor.

On page 3, in Column 2, under "Other Publications", Line 41, delete "107(5)2131-2137" and insert -- 107(5):2131-2137 --, therefor.

On page 3, in Column 2, under "Other Publications", Line 53, delete "hemglobinuria,"" and insert -- hemoglobinuria," --, therefor.

On page 3, in Column 2, under "Other Publications", Line 53, delete "Hermal." and insert -- Hematol. --, therefor.

On page 3, in Column 2, under "Other Publications", Line 64, delete "17:125-132" and insert -- 77:125-132 --, therefor.

On page 3, in Column 2, under "Other Publications", Line 70, delete "iocturnal" and insert -- nocturnal --, therefor.

On page 4, in Column 2, under "Other Publications", Line 6, delete "modem" and insert -- modern --, therefor.

On page 4, in Column 2, under "Other Publications", Line 15, delete "Medelian" and insert -- Mendelian --, therefor.

In the Specification

In Column 1, Line 23, delete "Seq _Listing.txt" and insert -- Seq_Listing.txt --, therefor.

In Column 1, Lines 45-46, delete "heomoglobinuria" and insert -- hemoglobinuria --, therefor.

In Column 1, Line 66, delete "II" and insert -- III --, therefor.

In Column 4, Line 2, delete "antibodies" and insert -- antibodies, --, therefor.

In Column 4, Line 43, delete "NO:5," and insert -- NO: 5, --, therefor.

In Column 4, Line 43, delete "NO:6," and insert -- NO: 6, --, therefor.

In Column 4, Line 44, delete "NO:7," and insert -- NO: 7, --, therefor.

In Column 4, Line 47, delete "NO:8," and insert -- NO: 8, --, therefor.

In Column 4, Line 47, delete "NO:9," and insert -- NO: 9, --, therefor.

In Column 4, Line 47, delete "NO:10." and insert -- NO: 10. --, therefor.

In Column 5, Line 45, delete "Instrument" and insert -- Instrument. --, therefor.

In Column 6, Line 33, delete "README.htm-1)" and insert -- README.html) --, therefor.

In Column 7, Line 49, delete "Neisseia" and insert -- Neisseria --, therefor.

In Column 7, Line 57, delete "scrum" and insert -- serum --, therefor.

In Column 7, Line 57, delete "T," and insert -- I, --, therefor.

In Column 8, Line 48, delete "at" and insert -- et --, therefor.

In Column 8, Line 53, delete “Measurer” and insert -- Measures --, therefor.

In Column 9, Line 51, delete “if” and insert -- life --, therefor.

In Column 10, Line 28, delete “(RNAI)” and insert -- (RNAi) --, therefor.

In Column 10, Line 54, delete “Antibodies” and insert -- antibodies --, therefor.

In Column 11, Line 32, delete “2d” and insert -- 2nd --, therefor.

In Column 12, Line 8, delete “anaphylatoxic” and insert -- anaphylatoxin --, therefor.

In Column 12, Line 22, delete “at” and insert -- et --, therefor.

In Column 12, Line 30, delete “at” and insert -- et --, therefor.

In Column 12, Line 41, delete “immunopharmaccticals” and insert -- immunopharmaceuticals --, therefor.

In Column 13, Line 15, delete “F(ab')2” and insert -- $F(ab')_2$ --, therefor.

In Column 13, Line 45, delete “at” and insert -- et --, therefor.

In Column 13, Line 56, delete “at” and insert -- et --, therefor.

In Column 14, Line 15, delete “(see.” and insert -- (see, --, therefor.

In Column 14, Line 29, delete “antibodies”” and insert -- antibodies --, therefor.

In Column 15, Line 1, delete “prepared,” and insert -- prepared --, therefor.

In Column 15, Line 5, after “6,331,415;” insert -- U.S. Pat. No. --.

In Column 15, Line 10, delete “at” and insert -- et --, therefor.

In Column 15, Line 17, delete “fill-length” and insert -- full-length --, therefor.

In Column 15, Line 38, delete “at” and insert -- et --, therefor.

In Column 15, Line 39, delete “at” and insert -- et --, therefor.

In Column 15, Line 40, delete “at” and insert -- et --, therefor.

In Column 15, Line 42, delete “Method” and insert -- Methods --, therefor.

In Column 16, Line 6, delete “pet” and insert -- per --, therefor.

In Column 18, Line 41, delete "aid" and insert -- and --, therefor.

In Column 18, Line 59, after "(mg/mL)]" insert -- . --.

In Column 19, Line 31, delete "farther" and insert -- further --, therefor.

In Column 19, Line 35, delete "needle-lees" and insert -- needle-less --, therefor.

In Column 20, Line 2, delete "meningoooccal" and insert -- meningococcal --, therefor.

In Column 20, Line 8, delete "prior-12-month" and insert -- prior 12-month --, therefor.

In Column 20, Line 14, delete "PBRC" and insert -- PRBC --, therefor.

In Column 20, Line 25, delete "level≥11.5" and insert -- level≥1.5 --, therefor.

In Column 21, Line 24, delete "HI" and insert -- III --, therefor.

In Column 21, Line 63, delete "Pharmokinetics" and insert -- Pharmacokinetics --, therefor.

In Column 22, Line 18, delete "2418.9*140.3" and insert -- 2418.9±140.3 --, therefor.

In Column 22, Line 50, delete "28.1±12.0%" and insert -- 28.1±2.0% --, therefor.

In Column 27, Lines 41-42, delete "functioning" and insert -- functioning, --, therefor.

In Column 28, Line 3, delete "8" and insert -- a --, therefor.

In Column 28, Line 21, after "independent" insert -- . --.

In Column 28, Line 60, delete "Bemshaw" and insert -- Bernshaw --, therefor.

In Column 29, Line 24, delete "Celia" and insert -- Cella --, therefor.

In Column 29, Line 52, after "Abstract" insert -- . --.

In Column 30, Line 2, delete "Patients." and insert -- Patients --, therefor.

In Column 30, Line 25, delete
"PKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH**AN**KTKPRE" and insert
-- PKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH**NA**KTKPRE --, therefor.

In Column 30, Line 27, delete
"P**EPE**QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY and insert
-- P**REP**QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY --, therefor.

In Column 30, Line 34, delete
"MDMRVPAQLLGLLLL**SLR**GARCDIQMTQSPSSLSASVGDRVTITCGASE" and insert
-- MDMRVPAQLLGLLLL**WLR**GARCDIQMTQSPSSLSASVGDRVTITCGASE --, therefor.

In Column 30, Line 61, delete "Eculizmuab" and insert -- Eculizumab --, therefor.

Patent Trial and Appeal Board

A petition has been filed in Patent Number 9,718,880, Application Number 15/148839 on February 28, 2019.

The AIA Review Number is IPR2019-00740.

To view the documents filed in this petition, go to https://ptab.uspto.gov and Search for the AIA Review Number.

- Enter your search criteria on the "Search PTAB" page
- Type in the AIA Review Number or Patent Number
- You will need to answer the CAPTCHA to prove that you are not a robot.
- Click on the "Search" button
- The search results will appear identifying the AIA Review Number
- Click on the "View Documents" button
- A pop up window will appear with a list of documents
- Click on the "Download" button to download the document.

Questions regarding this notice should be directed to the Patent Trial and Appeal Board at 571-272-7822.

Trials@uspto.gov
571-272-7822

Paper 15
Entered: August 30, 2019

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

AMGEN INC.,
Petitioner,

v.

ALEXION PHARMACEUTICALS, INC.,
Patent Owner.

IPR2019-00740
Patent 9,718,880 B2

Before TINA E. HULSE, ROBERT A. POLLOCK, and RYAN H. FLAX, *Administrative Patent Judges*.

POLLOCK, *Administrative Patent Judge.*

DECISION
Institution of *Inter Partes* Review
35 U.S.C. § 314(a)

I. INTRODUCTION

Amgen Inc. ("Petitioner") filed a Petition for an *inter partes* review of claims 1–3 of U.S. patent No. 9,718,880 B2 ("the '880 patent," Ex. 1001). Paper 2 ("Pet."). Alexion Pharmaceuticals ("Patent Owner" or "Alexion") timely filed a Preliminary Response. Paper 10 ("Prelim. Resp."). The parties further submitted an authorized Reply and Sur-Reply to the Preliminary Response. Paper 13 ("Reply"); Paper 14 ("Sur-reply").

We review the Petition, Preliminary Response, Reply, Sur-Reply, and accompanying evidence under 35 U.S.C. § 314. An *inter partes* review may not be instituted unless "the information presented in the petition . . . and any response . . . shows that there is a reasonable likelihood that the petitioner would prevail with respect to at least 1 of the claims challenged in the petition." 35 U.S.C. § 314(a). Further, a decision to institute may not institute on fewer than all claims challenged in the petition. *SAS Inst., Inc. v. Iancu*, 138 S. Ct. 1348, 1359–60 (2018).

After considering the evidence and arguments presented in the Petition and Preliminary Response, Reply, and Sur-Reply, we determine that Petitioner demonstrates a reasonable likelihood of prevailing in showing that at least one of the challenged claims of the '880 patent is unpatentable. Accordingly, we institute an *inter partes* review as to all the challenged claims of the '880 patent on all the grounds of unpatentability set forth in the Petition.

A. Real Parties-in-Interest

Petitioner identifies only itself as the real party-in-interest. Pet. 70. Patent Owner, likewise, identifies only itself as the real party-in-interest. Paper 3, 2.

B. Related Proceedings

The '880 patent shares essentially the same specification with U.S. Patent Nos. 9,725,504 B2 ("the '504 patent) and 9,732,149 ("the '149 patent"). Amgen has filed Petitions for *Inter Partes* Review of the '504, '880, and '149 patents in IPR2019-00739, IPR2019-00740, and IPR2019-00741, respectively. Pet. 70; Paper 3, 2.

The '504, '880, and '149 patents are related as follows: The '149 patent issued from application No. 15,284,015, filed January 19, 2017, which is a continuation of application No. 15/260,888 (now the '504 patent), filed on September 9, 2016, which is a continuation of application No. 15/148,839 (now the '880 patent), filed on May 6, 2016, which is a continuation of application No. 13/426,973, filed on March 22, 2012, which is a continuation of application No. 12/225,040, filed as application No. PCT/US2007/006606 on March 15, 2007. The parties do not dispute that March 15, 2007, is the relevant priority date of the challenged patent.

C. The '880 patent and Relevant Background

The '880 patent relates to the use of a humanized anti-C5 antibody (eculizumab) for the treatment of paroxysmal nocturnal hemoglobinuria. *See* Ex. 1001, Abstract. For reference, we reproduce Figure 1 from

paragraph 26 of the Balthasar Declaration,[1] illustrating the basic structure of an antibody such as eculizumab:

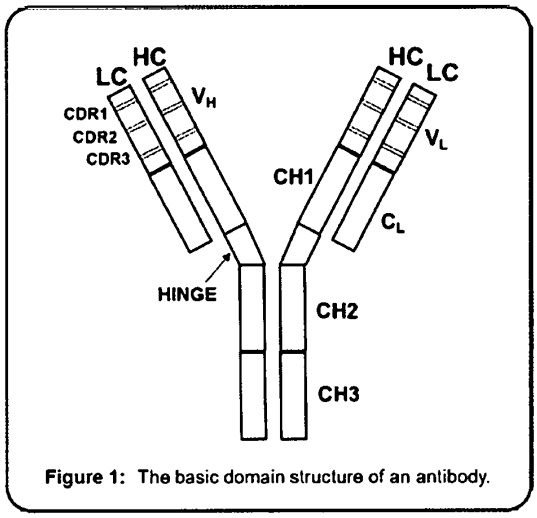


Figure 1: The basic domain structure of an antibody.

Figure 1 shows a basic antibody structure having hinged heavy chains (HC) and accompanying light chains (LC), each having constant regions (C_H and C_L) and variable regions (V_H and V_L), all arranged in a general "Y" shaped structure, as the variable regions and portions of the constant heavy chain regions are hinged away from one another. Ex. 1002 ¶¶ 23–25. The variable regions of each chain also include three complementarity determining regions (CDR), which provide the antibody with antigen-binding specificity. *Id.* ¶ 25.

---

[1] Declaration of Dr. Joseph P. Balthasar, Ph.D. (Ex. 1002, "Balthasar Declaration").

Paroxysmal nocturnal hemoglobinuria or PNH is an acquired hemolytic disease resulting from loss of function in certain cytoprotective proteins. *Id.* at 1:27–35; Ex. 1002 ¶¶ 32–33. This loss of function renders red blood cells, platelets and other blood cells highly sensitive to attack via activated complement proteins (explained in detail below). *Id.* The resultant complement-mediated lysis of blood cells results in anemia, hemoglobinuria, and related symptoms, which impair a patient's quality of life to the extent that "[m]any PNH patients depend on blood transfusions to maintain adequate erythrocyte hemoglobin levels." Ex. 1001, 1:42–53. As further explained by Petitioner's expert, Dr. Balthasar, "[a]s a result of the destruction of RBCs and the resultant release of free hemoglobin into the blood, 'PNH is characterized by hemolytic anemia (a decreased number of red blood cells), hemoglobinuria (the presence of hemoglobin in the urine particularly evident after sleeping), and hemoglobinemia (the presence of hemoglobin in the bloodstream.')" Ex. 1002 ¶ 33 (quoting Ex. 1005 ¶ 7).

"Complement" is a "system of plasma proteins . . . so-named because it complements the activity of antibody in the lysis of bacteria." Ex. 1022 R259; *see generally* Ex. 1001, 7:6–8:56. As part of the immune system, complement "has a central role in host defense against many micro-organisms and in the modulation of inflammatory reactions.'" *Id.*; *see* Ex. 1002 ¶ 29. The figure below, reproduced at paragraph 30 of the

Balthasar Declaration, shows "the main pathways and components of the complement activation system." Ex. 1022, R259.

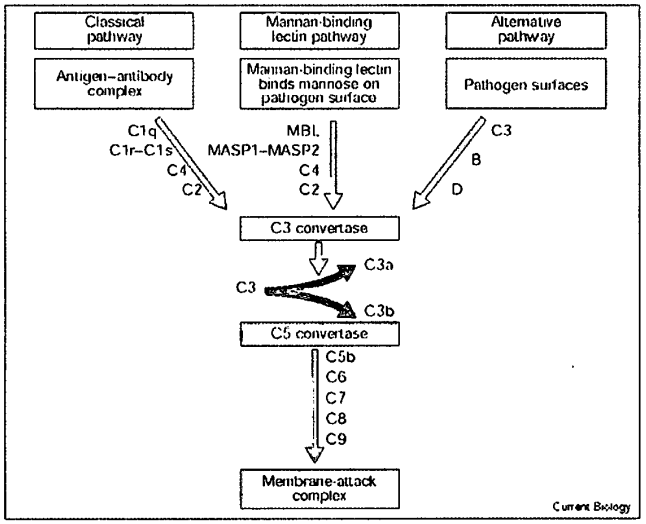


Figure 2: The main pathways and components of the complement system.

The above figure illustrates how various complement proteins are organized into the "classical," "mannan-binding," and "alternative" activation pathways. Ex. 1022, R259; *see* Ex. 1001, 7:17–19. All three pathways lead to the cleavage of C3 convertase and the resultant cleavage of C5 convertase into C5a and C5b. Ex. 1022, Fig. 1; *see* Ex. 1002 ¶ 30. As also summarized in the Balthasar Declaration, cleavage of C5 initiates the terminal complement cascade. Ex. 1002 ¶ 30. Conversely, blocking the cleavage of C5 prevents complement activation. *See, e.g.*, Ex. 1001, 10:57–65 ("U.S. Pat. No. 6,353,245 [Evans][2] teaches an antibody which binds to C5 and

[2] US 6,355,245 B1, issued Mar. 12, 2002 (Ex. 1007).

inhibits cleavage into C5a and C5b thereby decreasing the formation not only of C5a but also the downstream complement components."); 12:6–10.

According to the Specification of the '880 patent, eculizumab is a humanized monoclonal antibody directed against the terminal complement protein C5 convertase and is, thus, intended to suppress the terminal activation cascade and resultant complement activation. Ex. 1001, Abstract, 1:56–57 (citing Thomas C. Thomas et al., *Inhibition of Complement Activity by Humanized Anti-C5 Antibody and Single-Chain Fv*, 33(17) MOL. IMMUNOL. 1389–401 (1996) (Ex. 1023, "Thomas")). More specifically, "eculizumab" refers to humanized antibodies derived from mouse antibody 5G1.1, sometimes referred to as "murine 5G1.1" or "m5G1.1." *See* Prelim. Resp. 12–13. The term "humanized" refers to an antibody having a human framework, into which CDR regions from a non-human monoclonal antibody (*e.g.*, mouse) are inserted. Ex. 1007, 5:57–67; Ex. 1002 ¶¶ 27. Accordingly, humanized versions of non-human antibodies may be indicated by the prefix "h" or "hu" as in "h5G1.1" and "hu5G1.1." *See e.g.*, Pet. 11, 13, 14, n.9; Prelim. Resp. 12; Ex. 1002 ¶ 46.

Claim 1 refers to the amino acid sequences of SEQ ID NO: 2 and SEQ ID NO: 4, which together comprise an eculizumab antibody. *See generally* Prelim. Resp. 1–2. Thus, for example, the '880 patent identifies SEQ ID NO: 2 and SEQ ID NO: 4 as the "Eculizumab Heavy Chain" and "Eculizumab Light Chain," respectively. Ex. 1001, 30:14–31, 39–46 (subject to Certificate of Correction dated October 24, 2017). It is undisputed that SEQ ID NO: 2 encodes a hybrid IgG2/IgG4 heavy chain (i.e., having a genetically engineered heavy chain constant region derived

from portions of IgG2 and IgG4 isotype antibodies). *See, e.g.*, Pet. 3; Prelim. Resp. 14–16; Ex. 1033, 1258 (Figure 2). Eculizumab is the non-proprietary name for Alexion's SOLIRIS product, which was approved by the FDA "for treatment of patients with PNH." *See, e.g.,* Prelim. Resp. 1–2, 6–8 (citing Ex. 1033, 1256;[3] Ex. 2005, 1[4]); Pet. 2–3 (citing Ex. 1009, 2); *see also* Prelim. Resp. 16 ("The '880 patent claims recite the complete amino acid sequence for SOLIRIS® . . . the heavy chain consisting of SEQ ID NO: 2, and the light chain consisting of SEQ ID NO: 4. (AMG1001 at cols. 31-33, 35.).)"

The '880 patent also discusses the conduct and results of the TRIUMPH trial in which 88 red blood cell transfusion dependent PNH patients were randomly assigned "to receive either placebo or Eculizumab (Soliris™, Alexion Pharmaceuticals, Inc.)." *Id.* at 19:411–28:31.

> Study medication was dosed in a blinded fashion as follows: 600 mg eculizumab for patients randomly assigned to active drug, or placebo for those patients randomly assigned to placebo, respectively via IV infusion every 7± 1 days for 4 doses; followed by 900 mg eculizumab, or placebo, respectively, via IV infusion 7±1 day later; followed by a maintenance dose of 900 mg eculizumab, or placebo, respectively, via IV infusion every 14±2 days for a total of 26 weeks of treatment.

Id. at 20:29–40. The Specification concludes that "[t]he results of the TRIUMPH study indicate that terminal complement inhibition with

---

[3] Rother et al., *"Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria,"* 25(11) NATURE BIOTECHNOLOGY 1256–64 (2007).
[4] SOLIRIS Product Label (rev. 3/2007).

eculizumab safely and effectively addresses an important consequence of the underlying genetic defect in PNH hematopoietic stem cells by providing a therapeutic replacement for the terminal complement inhibitor deficiency." *Id.* at 28:26–31. "[E]culizumab stabilized hemoglobin levels, decreased the need for transfusions, and improved quality of life in PNH patients via reduced intravascular hemolysis." *Id.* at Abstract.

D. Challenged Claims

Petitioner challenges claims 1–3 of the '880 patent, which read as follows:

> 1. A pharmaceutical composition for use in treating a patient afflicted with paroxysmal nocturnal hemoglobinuria (PNH), wherein the composition is a sterile, preservative free, 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml antibody solution, wherein the antibody comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4.
>
> 2. A pharmaceutical composition comprising an anti-C5 antibody, wherein the anti-C5 antibody comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4.
>
> 3. The pharmaceutical composition of claim 2, wherein the pharmaceutical composition is a sterile, preservative free 300 mg single-use dosage form.

Ex. 1001, 39:2–16.

E. Asserted Grounds of Unpatentability

Petitioner asserts six grounds of unpatentability (Pet. 22–23):

Ground	Claim(s)	Basis	Asserted Reference(s)
1	2	102(b)[5]	Hillmen[6]
2	2	102(b)	Hill '05[7]
3	1 and 3	103(a)	Hillmen, Bell,[8] and Wang[9]
4	1 and 3	103(a)	Hill '05, Bell, and Wang
5	1–3	103(a)	Bell, Bowdish, [10] Evans, [11] and Wang
6	1–3	103(a)	Bell, Evans, Mueller[12], and Wang

[5] The Leahy-Smith America Invents Act, Pub. L. No. 112-29, 125 Stat. 284 (2011) ("AIA"), amended 35 U.S.C. §§ 102 and 103. Because the challenged claims of the '880 patent have an effective filing date before the effective date of the applicable AIA amendments, we refer to the pre-AIA versions of 35 U.S.C. §§ 102 and 103 throughout this Decision.
[6] Hillmen et al., "*Effect of Eculizumab on Hemolysis and Transfusion Requirements in Patients with Paroxysmal Nocturnal Hemoglobinuria,*" 350(6) N. ENGL. J. MED. 552–59 (2004) (Ex. 1004).
[7] Anita Hill et al., *Sustained response and long-term safety of eculizumab in paroxysmal nocturnal hemoglobinuria*, 106 BLOOD 2559–65 (2005) (Ex. 1047).
[8] Bell et al., US 2005/0191298 A1, published Sept. 1, 2005 (Ex. 1005).
[9] Wang, US 2005/0271660 Al, published Dec. 8, 2005 (Ex. 1028)
[10] Bowdish et al., US 2003/0232972 A1, published Dec. 18, 2003 (Ex. 1006).
[11] Evans et al., US 6,355,245 B1, issued March 12, 2002 (Ex. 1007).
[12] Mueller et al., WO 97/11971, published April 3, 1997 (Ex. 1008).

In support of its patentability challenges, Petitioner relies on, *inter alia*, the Declaration of Joseph P. Balthasar, Ph.D. Ex. 1002.

F. Overview of Asserted References

1. Overview of Hillmen (Ex. 1004)

Hillmen discloses that "[p]aroxysmal nocturnal hemoglobinuria (PNH) arises from a somatic mutation of the PIG-A gene in a hematopoietic stem cell and the subsequent production of blood cells with a deficiency of surface proteins that protect cells from attack by the complement system." Ex. 1004, 552.

> Patients with PNH have chronic, often disabling symptoms of fatigue and intermittent episodes of dysphagia, abdominal pain, and hemoglobinuria. These symptoms are thought to be related to the intravascular destruction of PNH type III erythrocytes, which are deficient in complement inhibitors, by autologous complement. The hemolytic anemia frequently renders the patients transfusion-dependent. In addition, patients have an extremely high risk of potentially life-threatening thrombosis, particularly thrombosis of the hepatic and cerebral veins. Approximately 50 percent of patients with PNH die of the disease; the median duration of survival after diagnosis is 10 years.

Id. at 557.

Hillmen reports on the efficacy of eculizumab for "reduc[ing] the incidence of intravascular hemolysis, hemoglobinuria, and transfusion requirements in patients with PNH." *Id.* at 553. "Eleven transfusion-dependent patients with PNH received infusions of eculizumab (600mg) every week for four weeks, followed one week later by a 900-mg dose and then by 900 mg every other week through week 12" *Id.* at Abstract, *see id.*

at 554. Based on this treatment, Hillmen concludes:

> Eculizumab is safe and well tolerated in patients with PNH. This antibody against terminal complement protein C5 reduces intravascular hemolysis, hemoglobinuria, and the need for transfusion, with an associated improvement in the quality of life in patients with PNH.

Id. at Abstract.

Hillmen characterizes eculizumab as "a recombinant humanized monoclonal antibody that was designed to block the activation of terminal complement components." *Id.* (citing, e.g., Thomas (Ex. 1023)). According to Hillmen, the antibody "binds specifically to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b, thereby preventing the release of the inflammatory mediator C5a and the formation of the cytolyticpore C5b-C9." *Id.* Because eculizumab "specifically prevents cleavage of C5, which is necessary for assembly of the membrane-attack complex," the treatment "presumably prolongs the survival of type III erythrocytes . . . which are highly sensitive to lysis by complement." *Id.* at 557.

2. Overview of Hill '05 (Ex. 1047)

Hill '05 reports the results of a follow up study to Hillmen (reference 2) "to assess the long-term efficacy and safety of patients with PNH." Ex. 1047, 2559. "After completion of an initial 12-week study [described in Hillmen], all patients chose to participate in the 52-week extension study. Eculizumab, administered at 900 mg every 12 to 14 days, was sufficient to completely and consistently block complement activity in all patients." *Id.* at Abstract.

3. Overview of Bell (Ex. 1005)

Bell discloses the treatment of PNH "using a compound which binds to or otherwise blocks the generation and/or activity of one or more complement components . . . . In particularly useful embodiments, the compound is an anti-C5 antibody selected from the group consisting of h5G1.1-mAb (eculizumab), h5G1.1-scFv (pexelizumab) and other functional fragments of h5G1.1." *Id.* ¶ 12; *see also id.* ¶ 52 ("The antibody h5G1.1-mAb is currently undergoing clinical trials under the tradename eculizumab."). Bell further discloses: "Methods for the preparation of h5G1.1-mAb, h5G1.1-scFv and other functional fragments of h5G1.1 are described in [Evans] and [Thomas] . . . the disclosures of which are incorporated herein in their entirety." *Id.* ¶ 52.

As noted by Dr. Balthasar, the data disclosed in Bell overlaps with that in Hillmen, but further includes data on extension studies in which patients continued treatment for paroxysmal nocturnal hemoglobinuria for a total of two additional years. *See* Ex. 1002 ¶ 40 (citations omitted).[13] Briefly, eleven transfusion-dependent PNH patients received weekly 600 mg doses of eculizumab by infusion for four weeks, followed by "900 mg of eculizumab 1 week later[,] then 900 mg on a bi-weekly basis." Ex. 1005

---

[13] Page 37, footnote 16, of the Petition states: "Because the clinical study taught in Bell is the same C02-001 study disclosed in Hillmen, which discloses the eculizumab amino acid sequences of SEQ ID NOs: 2 and 4, Bell, too, therefore anticipates claims 1–4 and 7–10 for the same reasons discussed above for Hillmen." Despite this assertion, Petitioner's sole anticipation-based Grounds in this IPR are based on Hillmen (Ground 1) and Hill '05 (Ground 2). *See* Pet. 22–23.

¶¶ 81–82. Bell characterizes the first twelve weeks of treatment as a "pilot study." *Id.* ¶ 82. "Following completion of the initial acute phase twelve week study, all patients participated in an extension study conducted to a total of 64 weeks. Ten of the eleven patients participated in an extension study conducted to a total of two years." *Id.* Bell concludes that "[p]atients in the two year study experienced a reduction in adverse symptoms associated with PNH. *Id.* ¶¶ 82, 96.

4. Overview of Wang (Ex. 1028)

Wang discloses formulations of eculizumab suitable for nebulization and pulmonary delivery. *See, e.g.*, ¶¶ 25, 62, 67. Wang discloses formulations comprising from 1 to 30 mg/ml eculizumab, and provides evidence that a formulation having 30 mg/ml eculizumab can be effectively and efficiently delivered using a conventional nebulizer. *Id.* ¶¶ 171–173, Fig. 10.

5. Overview of Bowdish (Ex. 1006)

Bowdish discloses "[i]mmunoglobulins or fragments thereof hav[ing] a peptide of interest inserted into a complementarity determining region (CDR) of an antibody molecule," whereupon, "[t]he antibody molecule serves as a scaffold for presentation of the peptide and confers upon the peptide enhanced stability." Ex. 1006 ¶ 6. In certain "embodiments, the peptide replacing the amino acids of a CDR is an agonist TPO [thrombopoeitin] peptide." *Id.* ¶17.

In Example 4, Bowdish describes a TPO mimetic peptide graft into the heavy chain CDR3 of antibody framework 5G1.1, described in Evans,

which it incorporates by reference. *Id.* ¶¶ 191–193. According to Bowdish:

> Construction of 5G1.1 is described in U.S. Application. Ser. No. 08/487,283, incorporated herein by reference.[14] The sequence was cloned into 5G1.1 in such a fashion as to replace the native CDR3 . . . [wherein t]he peptide graft translated into amino acids is Leu Pro Ile Glu Gly Pro Thr Leu Arg Gln Trp Leu Ala Arg Ala Pro Val (SEQ. ID. NO: 66). The 5G1+peptide was produced as a whole IgG antibody (See FIGS. 13A and 13B).

Id. ¶ 191. "Purified 5G1.1+peptide antibody as well as the parental 5G1.1 were analyzed for their ability to bind to cMpl receptor by FACS analysis." *Id.* ¶ 192.

In SEQ ID NOs: 69 and 70, respectively, Bowdish discloses the amino acid and nucleotide sequences for the "5G1.1 Light Chain." In SEQ ID NO: 67, Bowdish discloses the amino acid sequence of the "5G1.1–TPO Heavy Chain," with the substituted TPO mimetic sequence marked in bold. An excerpt of that sequence showing the amino acids of the TPO substitution in bold reads: DTAVYYCAR**LPIEGPTLRQWLAARAPV** WGQGTLVTVSS. Bowdish discloses the corresponding nucleotide sequence in SEQ ID NO: 68.

6. Overview of Evans (Ex. 1007)

Evans discloses anti-C5 antibodies useful in the treatment of glomerulonephritis (GN). Ex. 1007, Abstract. Evans' Example 7 describes the isolation anti-C5 monoclonal antibodies from mouse hybridoma designated 5G1.1. *Id.* at 37:34–39:30. In Figures 18 and 19, respectively,

---

[14] U.S. Application. Ser. No. 08/487,283 matured into U.S. Patent No. 6,355,245 B1, referenced herein as Evans (Ex. 1007).

Evans discloses the amino acid sequence of the light and heavy chain variable regions of mouse antibody 5G1.1, with "[t]he complementarity determining region (CDR) residues according to the sequence variability definition or according to the structural variability definition . . . [bolded] and [underlined], respectively." *Id.* at 9:65–10:20. A representation of an excerpt of the heavy chain sequence showing the amino acids of CDR3 so marked reads: DSAVYYCAR**YFFGSSPNWYFD**VWGAGTTVTVSS.

Evans describes making a series of different humanized 5G1.1 scFv[15] and full-length antibodies containing the CDR regions from the murine 5G1.1 antibody. Ex. 1007, 37:35–39:30, 42:59–45:33. With respect to the former, Evans discloses that "[p]articularly preferred constant regions . . . are IgG constant regions, which may be unaltered, or constructed of a mixture of constant domains from IgGs of various subtypes, e.g., IgG1 and IgG4." *Id.* at 45:29–33.

In Example 11, Evans discloses steps in the humanization of mouse antibody 5G1.1, including the construction of recombinant antibody, 5G1.1 scFv CO12. *Id.* at 42:59–45:33. According to Dr. Balthasar, the construct "'5G1.1 scFv CO12" contains all six 5G1.1 CDR regions and its sequence aligns perfectly with the eculizumab variable regions claimed by the '880 patent." Ex. 1002 ¶ 55 (comparing portions of SEQ ID NO: 2 of the '880 patent with portions of Evans' SEQ ID NO: 2). Dr. Balthasar's Figure 6, for

---

[15] As Dr. Balthasar notes, "a scFv comprises an antibody's light and heavy chain variable domains connected by a linker." Ex. 1002 ¶ 54, n.4 (citing Ex. 1007, 6:39-41).

example, shows that both sequences contain the larger of the two overlapping amino acid sequences Evans identifies as CDR3, YFFGSSPNWYFDV. *Id.*

7. Overview of Mueller (Ex. 1008)

Mueller discloses "[a]ntibodies to porcine P-selecting protein, porcine VCAM protein and porcine CD86 protein are useful for diagnosing human rejection of porcine xenotransplants and for improving xenotransplantation of porcine, cells, tissues and organs into human recipients." Ex. 1008, Abstract. According to Mueller, one object of the invention is to provide antibody molecules that neither activate complement nor bind to the FC receptor. *Id.* at 7:28–31. To achieve these and other goals, Mueller points to "[r]ecombinant (chimeric and/or humanized) antibody molecules comprising the C1 and hinge regions of human IgG2 and the C2 and C3 regions of human IgG4, such antibodies being referred to hereinafter as 'HuG2/G4 mAb.'" *Id.* at 8:23–26. Mueller developed and tested "chimeric antibodies containing the C1 and hinge region of human IgG2 and the C2 and C3 regions of human IgG4." *Id.* at 12:19–33. As controls for these experiments, Mueller used "a humanized antibody directed against human C5 (h5G1.1 C012 HuG4 mAb)." *Id.* at 11:34–12:4. 12:34–13:2, Figures 11, 12, 15. On pages 58–61 of the reference, Mueller discloses the cDNA and amino acid sequence of "Human G2/G4."

II. ANALYSIS

A. Principles of Law

"In an [*inter partes* review], the petitioner has the burden from the onset to show with particularity why the patent it challenges is unpatentable." *Harmonic Inc. v. Avid Tech., Inc.*, 815 F.3d 1356, 1363 (Fed. Cir. 2016) (citing 35 U.S.C. § 312(a)(3) (requiring *inter partes* review petitions to identify "with particularity . . . the evidence that supports the grounds for the challenge to each claim")). This burden of persuasion never shifts to Patent Owner. *See Dynamic Drinkware, LLC v. Nat'l Graphics, Inc.*, 800 F.3d 1375, 1378 (Fed. Cir. 2015) (discussing the burden of proof in *inter partes* review).

To establish anticipation, each and every element in a claim, arranged as recited in the claim, must be found in a single prior art reference. *Net MoneyIN, Inc. v. VeriSign, Inc.*, 545 F.3d 1359, 1369 (Fed. Cir. 2008); *Karsten Mfg. Corp. v. Cleveland Golf Co.*, 242 F.3d 1376, 1383 (Fed. Cir. 2001). This "single prior art reference must expressly or inherently disclose each claim limitation." *Finisar Corp. v. DirecTV Group, Inc.*, 523 F.3d 1323, 1334 (Fed. Cir. 2008). "Under the principles of inherency, if the prior art necessarily . . . includes[ ] the claimed limitations, it anticipates." *MEHL/Biophile Int'l Corp. v. Milgraum*, 192 F.3d 1362, 1365 (Fed. Cir. 1999). Similarly, "[a] reference anticipates a claim if it discloses the claimed invention 'such that a skilled artisan could take its teachings in combination with his own knowledge of the particular art and be in possession of the invention.'" *In re Graves*, 69 F.3d 1147, 1152 (Fed. Cir. 1995) (internal citation and emphasis omitted). Moreover, "it is proper to

take into account not only specific teachings of the reference but also the inferences which one skilled in the art would reasonably be expected to draw therefrom." *In re Preda*, 401 F.2d 825, 826 (CCPA 1968); *see Eli Lilly v. Los Angeles Biomedical Res. Inst.*, 849 F.3d 1073, 1074–75 (Fed. Cir. 2017).

A claim is unpatentable under 35 U.S.C. § 103(a) if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which that subject matter pertains. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 406 (2007). The question of obviousness is resolved on the basis of underlying factual determinations including: (1) the scope and content of the prior art; (2) any differences between the claimed subject matter and the prior art; (3) the level of ordinary skill in the art; and (4) objective evidence of non-obviousness. *Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966).

In analyzing the obviousness of a combination of prior art elements, it can be important to identify a reason that would have prompted one of skill in the art "to combine . . . known elements in the fashion claimed by the patent at issue." *KSR*, 550 U.S. at 418. A precise teaching directed to the specific subject matter of a challenged claim is not necessary to establish obviousness. *Id.* Rather, "any need or problem known in the field of endeavor at the time of invention and addressed by the patent can provide a reason for combining the elements in the manner claimed." *Id.* at 420. Accordingly, a party that petitions the Board for a determination of unpatentability based on obviousness must show that "a skilled artisan

would have been motivated to combine the teachings of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success in doing so." *In re Magnum Oil Tools Int'l, Ltd.*, 829 F.3d 1364, 1381 (Fed. Cir. 2016) (internal quotations and citations omitted).

B. Person of Ordinary Skill in the Art

In determining the level of skill in the art, we consider the type of problems encountered in the art, the prior art solutions to those problems, the rapidity with which innovations are made, the sophistication of the technology, and the educational level of active workers in the field. *Custom Accessories, Inc. v. Jeffrey-Allan Indus. Inc.*, 807 F.2d 955, 962 (Fed. Cir. 1986); *Orthopedic Equip. Co. v. United States*, 702 F.2d 1005, 1011 (Fed. Cir. 1983).

Petitioner contends that a person of ordinary skill in the art as of the relevant date would have "had an M.D. and/or a Ph.D. in immunology, biochemistry, cell biology, molecular biology, pharmaceutics, or a related discipline, with at least two years of experience in the field." Pet. 21–22. Patent Owner does not dispute this definition, but suggests that we interpret the relevant field as directed to "engineering monoclonal antibodies for human therapeutic use, either in the laboratory or industry." *See* Prelim. Resp. 43 (emphasis omitted); *see also id.* (arguing that Petitioner cannot prove unpatentability of the challenged claims under either party's definition).

Considering that each of the asserted references (discussed *infra*) is substantially directed to the development of monoclonal antibodies for the

treatment or diagnosis of human disease, Patent Owner's proposed clarification provides useful context. *See Okajima v. Bourdeau*, 261 F.3d 1350, 1355 (Fed. Cir. 2001) ("the prior art itself [may] reflect[] an appropriate level" as evidence of the ordinary level of skill in the art) (quoting *Litton Indus. Prods., Inc. v. Solid State Sys. Corp.*, 755 F.2d 158, 163 (Fed. Cir. 1985)). Accordingly, we define one of ordinary skill in the art as a person with an M.D. and/or a Ph.D. in immunology, biochemistry, cell biology, molecular biology, pharmaceutics, or a related discipline, with at least two years of experience in engineering monoclonal antibodies for human therapeutic use, either in the laboratory or industry. Our decision whether to institute, however, does not turn on which party's definition of the skilled artisan is used, and our determinations would be unchanged if we applied Petitioner's definition.

C. Claim Construction

Based on the filing date of the Petition (Feb. 28, 2019), the Board interprets claim terms in an *inter partes* review ("IPR") using the same claim construction standard that is used to construe claims in a civil action in federal district court. *See* 83 Fed. Reg. 51,340 (Nov. 13, 2018) (to be codified at 37 C.F.R. pt. 42).

In construing claims, district courts give claims their ordinary and customary meaning, which is "the meaning that the term would have to a person of ordinary skill in the art in question at the time of the invention." *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312–13 (Fed. Cir. 2005) (en banc). Sources for claim interpretation include "the words of the claims themselves, the remainder of the specification, the prosecution history [i.e., the intrinsic

evidence], and extrinsic evidence concerning relevant scientific principles, the meaning of technical terms, and the state of the art." *Id.* at 1314 (quoting *Innova/Pure Water, Inc. v. Safari Water Filtration Sys., Inc.*, 381 F.3d 1111, 1116 (Fed. Cir. 2004)). "[T]he claims themselves [may] provide substantial guidance as to the meaning of particular claim terms." *Id.* However, the claims "do not stand alone," but are part of "'a fully integrated written instrument,' consisting principally of a specification that concludes with the claims," and, therefore, the claims are "read in view of the specification." *Id.* at 1315 (quoting *Markman v. Westview Instruments, Inc.*, 52 F.3d 967, 978–79 (Fed. Cir. 1995)).

Neither party requests the construction of any claim term. *See, e.g.*, Pet. 21 (citing Ex. 1002 ¶ 64). At this stage in the proceeding, we need only construe the claims to the extent necessary to determine whether to institute *inter partes* review. *See Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co.*, 868 F.3d 1013, 1017 (Fed. Cir. 2017) ("[W]e need only construe terms 'that are in controversy, and only to the extent necessary to resolve the controversy.'" (quoting *Vivid Techs., Inc. v. Am. Sci. & Eng'g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999))). At this stage in the proceeding we find it unnecessary to construe the language of any challenged claim because the claim language is readily understandable on its face, within the context of the claims, to the person of ordinary skill in the art.

With this understanding of the claims, as well as the legal principles and our understanding of the definition of the skilled artisan as set forth above in mind, we address the parties' positions below.

D. Anticipation by Hillmen (Ground 1)

In Ground 1, Petitioner challenges claim 2 as anticipated by Hillmen. Pet. 25–31. Patent Owner opposes. Prelim. Resp. 44–51.

1. The Parties' Contentions

Petitioner contends "Hillmen discloses all the limitations of claim 2, either expressly or inherently, and is enabling." Pet. 25 (citing Ex. 1002 ¶¶ 73–81). In particular, Petitioner argues that "Hillmen expressly disclosed a 'pharmaceutical composition comprising an antibody that binds C5' as claimed because Hillmen disclosed administering infusion of *eculizumab* formulations to patients, and eculizumab was a known antibody that binds C5. *Id.* at 26–27 (citing Ex. 1004, Abstract). But, with respect to that amino acid sequence of the claimed heavy and light chains, Petitioner contends "Hillmen's antibody necessarily 'comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4' because Alexion admitted that Hillmen's eculizumab possesses those very amino acid sequences." *Id.* at 26–27. In particular, Petitioner argues that:

> During prosecution, Alexion submitted a list of eculizumab clinical studies—including Hillmen's Phase 2 Pilot Study (Study "C02-001")—and stated that "the antibody (eculizumab) used in each of the studies ... *contained the heavy and light chain sequences of SEQ ID NOs: 2 and 4*." AMG1014, 767(¶6); *see also, id.*, 765 (study number "C02-001").

Id. at 26.

Although Petitioner does not argue that Hillmen literally and expressly disclosed the claimed antibody structure with SEQ ID NOs: 2 and 4 (it does not), Petitioner's position is that, because Hillmen disclosed a trial

of the SOLIRIS eculizumab antibody, and because Patent Owner conceded that the eculizumab antibody used is the same anti-C5 antibody as claimed, that Hillmen inherently discloses the claimed sequences. Pet. 27–29 (citing *In re Crish*, 393 F.3d 1253 (Fed. Cir. 2004)).

Petitioner's inherency rationale, based on "the general knowledge in the relevant field" (*id.* at 30), is summarized as follows. Contemporaneous with Hillmen's disclosure, the skilled artisan would have known that Bowdish disclosed the entire amino acid sequence of eculizumab, but for a heavy chain CDR3 region, which Bowdish disclosed as substituted with a TPO (thrombopoietin) amino acid sequence; the skilled artisan, however, would have known that Evans disclosed the amino acid sequences of eculizumab's heavy and light chain variable domains, including heavy chain CDR3 region absent from Bowdish; alternatively, the skilled artisan would have understood that Mueller disclosed the hybrid IgG2/IgG4 heavy chain and light chain constant domains of eculizumab, whereas Evans disclosed the respective variable domains. Pet. 29–31 (citing Ex. 1004, 553–554; Ex. 1006 ¶¶ 191–193, Figs. 13A, 13B; Ex 1007, 44:4–13; Ex. 1008, 52–53, 58–61; Ex. 1002 ¶¶ 60, 78–80). With this knowledge in hand, the skilled artisan would have known that Hillman discloses a method of treatment using eculizumab—which was necessarily the claimed anti-C5 antibody with the claimed SEQ ID NOs: 2 and 4. *Id.*

Petitioner also appears to argue that Hillmen inherently discloses the hybrid IgG2/IgG4 heavy chain portion of SEQ ID NO: 2, because one of ordinary skill in the art would have known that eculizumab contains this structure. Pet. 13 (citing Ex. 1034, 1279; Ex. 1049, 838–839; Ex. 1002

¶¶ 47–50). Petitioner argues, for example, that Tacken explicitly describes eculizumab as "containing an '*IgG2/IgG4 constant region.*'" *Id.* at 13–14 (quoting Ex. 1034, 1279); *see, e.g.*, Ex. 1002 ¶ 47 ("Tacken confirms that eculizumab contains a hybrid IgG2/IgG4 constant region. AMG1034, 1278-1279. Tacken describes using 'h5G1.1-mAb (5G1.1, eculizamab [sic]; Alexion Pharmaceuticals)' containing an 'IgG2/IgG4 constant region.' AMG1034, 1279.").

Petitioner also relies on statements made by Patent Owner before the examiner during prosecution of U.S. Patent Application Ser. No. 11/127,438, where, in arguing that disclosures upon which the applicant relied for priority were supportive of the then-pending claims, Alexion stated:

> Applicant respectfully disagrees and asserts that the priority applications provide ample written support for the claimed descriptions. For example, the priority documents each describe that "Particularly useful anti-C5 antibodies are h5G1.1, h5G1.1-scFv and functional fragments of h5G1.1 are *described in U.S. Patent No. 6,355,245* [Evans], the disclosures of which are incorporated herein in their entirely [*sic*] by this reference . . . Applicant submits that h5G1.1 . . . [was] *well-known to one of ordinary skill in the art as eculizumab* . . . at the time of filing of priority applications.

See Pet. 10 (quoting Ex. 1049, 838–39 (emphasis Petitioner's)). Thus, Petitioner argues, Alexion represented to the Patent Office that the antibody structure disclosed in Evans was well-known to the skilled artisan so that such a person of skill would have considered this structure to be the eculizumab in Hillmen, and which was publically disclosed before the March 15, 2007 priority date of the '880 patent.

Patent Owner takes the position that "[p]rior to March 15, 2007, the priority date of the '880 patent, the unique amino acid sequence of SOLIRIS® was not publicly known or disclosed in the prior art." Prelim. Resp. 1.

> If a POSA were searching for the sequence of "eculizumab" as described in the art, the literature as of March 15, 2007 identified an amino acid sequence and corresponding structure that is very different from what the '804 patent claims. In particular, publications describing the safety, efficacy, and clinically relevant biological activity of "eculizumab" consistently directed a POSA to the 1996 "Thomas" publication (AMG1023) for the structure and design of the antibody, which in turn described a humanized antibody constructed with a naturally-occurring "IgG4" heavy chain constant region. The claimed antibody of the '880 patent has a very different, uniquely engineered, non-naturally occurring constant region that was nowhere described in Thomas or the prior art literature showing the safety and efficacy of "eculizumab."

Id. at 2–3 (emphasis omitted).

It is Patent Owner's position that, reading Hillmen's disclosure of "eculizumab" and Hillmen's reference to Thomas, the skilled artisan would not have been directed to the version of eculizumab encompassed by the challenged claims, but would have understood Hillmen to refer to Thomas's disclosed eculizumab, which is an IgG4 isotype antibody and does not have the hybrid IgG2/IgG4 heavy chain sequence as described in SEQ ID NO: 2. *Id.* at 3, 11–13 (referencing Ex. 1023 (Thomas)). In particular, Patent Owner notes that Hillmen cites Thomas as *the* reference for eculizumab. *Id.* at 3, 12 (citing Ex. 1004, 533 (which cites Ex. 1023 as reference "15")).

> Thomas . . . described the design and testing of a humanized anti-C5 antibody (termed "humanized 5G1.1" or "h5G1.1") featuring

> an "IgG4" heavy chain constant region, which was selected because the IgG4 isotype was thought to avoid activating human complement. (AMG1023 at 1396, 1399.) Thomas reported data showing that the IgG4 humanized antibody had suitable affinity and specificity, and was as effective as the original mouse antibody (termed "murine 5G1.1" or "m5G1.1") in an *in vitro* assay showing activity blocking C5 cleavage and preventing lysis of blood cells due to complement activity. (AMG1023 at 1396.)

Id. at 12–13 (emphasis omitted).

Patent Owner states that "[t]oday, but *not* prior to the March 15, 2007 priority date for the '880 patent, it is known that SOLIRIS® has the specific amino acid sequence recited in the claims of the '880 patent, namely, 'a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4.'" *Id.* at 15. Patent Owner argues that, as of the critical date, Hillmen did not

> enable[ ] a POSA to make and use the specific antibody recited in claims 1-3 and 7-10 without undue experimentation, because Hillmen guided a POSA as of March 15, 2007 to make and use a very different antibody – the ***IgG4*** isotype antibody of Thomas. *See, e.g.*, *Elan Pharm., Inc. v. Mayo Found. for Med. Educ. & Research*, 346 F.3d 1051, 1055 (Fed. Cir. 2003) (for a reference to anticipate, "[i]t is insufficient to name or describe the desired subject matter, if it cannot be produced without undue experimentation").

Id. at 45. Patent Owner further argues that

> [t]he mere naming of an investigational product (*e.g.*, "eculizumab") in a prior art publication does ***not*** inherently anticipate later-filed patent claims detailing the specific structure or composition of that product (*i.e.*, SEQ ID NOs: 2 and 4), if a POSA could not have ***necessarily*** determined the later claimed structure/composition from the information publicly available as

of the priority date.

Id. at 47 (citing *Endo Pharm. Solutions, Inc. v. Custopharm Inc.*, 894 F.3d 1374, 1378–83 (Fed. Cir. 2018)).

2. Analysis

At this stage in the proceedings and for the reasons discussed below, we find Petitioner has not carried its burden to show a reasonable likelihood of anticipation of claim 2 of the '880 patent under Ground 1.

Petitioner concedes Hillmen does not expressly disclose the claimed antibody; instead, Petitioner relies on the doctrine of inherency and a post-priority-date admission by Patent Owner that the pharmaceutical (eculizumab) referenced in Hillmen was actually the claimed antibody.

Hillmen "tested the clinical efficacy of eculizumab, a humanized antibody that inhibits the activation of terminal complement components, in patients with PNH." Ex. 1004, 552. Hillmen teaches that "[e]culizumab is a recombinant humanized monoclonal antibody that was designed to block the activation of terminal complement components.[14,15] It binds specifically to the terminal complement protein C5." *Id.* at 553. Citation "14" of Hillmen refers to Lutz Riechmann et al., *Reshaping human antibodies for therapy*, 332 NATURE 323–27 (1988). This reference is not an exhibit in this proceeding and Petitioner does not suggest that it mentions eculizumab. Citation "15" of Hillmen refers to Thomas (Ex. 1023), which discloses a monoclonal antibody (5G1.1) that recognizes the human complement protein C5, which was shown to effectively block C5 cleavage. *See* Ex. 1023, 1389.

Thomas discloses the process of developing a humanized antibody (h5G1.1 HuG4) for human C5. Thomas summarizes this work in a section

titled "*Construction of a humanized h5G1.1 antibody*":

> Having demonstrated the effective humanization of the 5G1.1 variable regions, an intact humanized antibody (IgG4 isotype) was constructed and produced in 293-EBNA cells. The avidity of this humanized antibody (h5G1.1 HuG4) for human C5, was compared to the murine 5G1.1 mAb by determining the ability of each to compete binding of biotinylated 5G1.1 mAb to C5 (Fig. 9). The humanized h5G1.1 mAb had a two-fold lower avidity than the murine antibody. However, the humanized h5G1.1 HuG4 antibody was equipotent with the murine antibody at protecting PAEC from lysis by human serum, with a 0.5-fold molar ratio of antibody to C5 (1:1 ratio of antibody binding sites to C5) completely inhibiting lysis of the PAEC (Fig. 10).

Id. at 1396.[16] We find nothing in Thomas that expressly discloses or alludes to a hybrid IgG2/IgG4 antibody. *See generally* Ex. 1023.

We also consider Dr. Balthasar's assertion that one of ordinary skill in the art "would have known that eculizumab contains a hybrid IgG2/IgG4 heavy chain constant region" because Tacken "describes using 'h5G1.1-mAb (5G1.1, eculizamab [sic]; Alexion Pharmaceuticals)' containing an 'IgG2/IgG4 constant region.'" Ex. 1002 ¶ 47 (citing Ex. 1034, 1279).

In addressing this assertion, Patent Owner takes the position that Tacken (also addressed in the context of Petitioner's obviousness challenges) is non-analogous art because it "does not involve the same field as the '880 patent, and would not have been reasonably pertinent to the

---

[16] We note Patent Owner's argument that as of the '880 patent's priority date, many other references cited Thomas when referring to eculizumab. *See* Prelim. Resp. 23–26, Table 1. Without going into detail, we find Patent Owner has accurately shown how other, contemporaneous prior art references cited Thomas for this purpose.

particular problem addressed by the '880 patent." Prelim. Resp. 36–37; Sur-Reply 2–3. On the present record, we do not find Patent Owner's argument persuasive. *See In re Geisler*, 116 F.3d 1465, 1470 (Fed. Cir. 1997) (mere lawyer's arguments and conclusory statements that are unsupported by factual evidence are entitled to little probative value).

"Prior art is analogous if it is from the same field of endeavor or if it is reasonably pertinent to the particular problem the inventor is trying to solve." *Circuit Check Inc. v. QXQ Inc.*, 795 F.3d 1331, 1335 (Fed. Cir. 2015). In the present case, Tacken discloses the development of "a humanized antibody, hD1V1G2/G4 (hD1), directed against the C-type lectin DC-specific intercellular adhesion molecule 3–grabbing nonintegrin (DC-SIGN) to explore its capacity to serve as a target receptor for vaccination purposes." Ex. 1034, Abstract. Given Patent Owner's position that the relevant field of the '880 patent encompasses the "engineering monoclonal antibodies for human therapeutic use, either in the laboratory or industry" (*see* Section II(B), above), Tacken's development of the monoclonal antibody hD1V1G2/G4 (hD1) "for vaccination purposes" appears to be in the same field of endeavor as the invention of the '880 patent. Moreover, because Tacken's antibodies employ the same IgG2/IgG4 constant region as SEQ ID NO: 2 of the asserted claims, we are unconvinced that Tacken is not reasonably pertinent to the '880 patent.

With reference to its hD1V1G2/G4 (hD1) antibody, Tacken discloses that, "[a]n isotype control antibody, h5G1.1-mAb (5G1.1, eculizumab; Alexion Pharmaceuticals) containing the same IgG2/IgG4 constant region, is specific for the human terminal complement protein C5." Ex. 1034, 1279.

Although, as Patent Owner points out, Tacken then cites to Thomas, Patent Owner does not address why Tacken would use an eculizumab having the IgG4 constant region as set forth in Thomas as "[a]n isotype control antibody" for one having an IgG2/IgG4 constant region. Dr. Balthasar, however, testifies that Tacken also cites Mueller II[17] "as providing information about the hybrid IgG2/IgG4 constant region portion of eculizumab." Ex. 1002 ¶ 56 (citations omitted); *see* Ex. 1034, 1279 (indicating that in the construction of hD1V1G2/G4 (hD1), "humanized variable heavy and variable light regions were [] genetically fused with a human hybrid IgG2/IgG4 constant domain," as set forth in Mueller II (reference 17)). On the present record, we read Tacken as disclosing that humanized monoclonal antibody 5G1.1, known as eculizumab, encompasses a molecule having a hybrid IgG2/IgG4 constant domain.

That Tacken, Bowdish, and Mueller suggest that eculizumab may include an IgG2/IgG4 constant domain, however, does not necessarily lead to the conclusion that Hillmen discloses the same molecule. Rather, the present record indicates that "eculizumab" is not a single chemical entity. To the contrary, the art identified as "eculizumab" at least two such compounds that differed in the heavy chain constant region: one having the IgG4 isotype (as evidenced in Thomas), and a second having a hybrid IgG2/IgG4 isotype (as evidenced by, e.g., Tacken).

---

[17] John P. Mueller et al., *Humanized porcine VCAM-specific monoclonal antibodies with chimeric IgG2/G4 constant regions block human leukocyte binding to porcine endothelial cells*, 34 Mol. Immunol. 441–52 (1997) (Ex. 1031).

Patent Owner made the above dichotomy explicit in an official representation to the Patent Office. During Alexion's prosecution of Application No. 11/127,438, an application outside of the chain of priority of the '880 patent, but claiming the use of eculizumab for the treatment of pulmonary conditions, the Examiner took the position that "the description of 'eculizumab or pexelizumab' as well as 'the mutated Fc portions' in the priority documents is not readily apparent." Ex. 1049, 741, 830–834 (representative claim amendments). In response, Alexion asserted that

> **eculizumab was first constructed in the IgG4 isotype**, see, e.g., the bridging paragraph of the left and right columns of page 1396 of Thomas et al. (1996) [Ex. 1023], . . . **and then into the G2/G4 format**, see Mueller et al. (1997) *Molecular Immunology,* Vol. 34, No.6, pages 441-452 [Ex. 1031], . . . while **in either form** the h5G1.1 antibody was well known to be incapable to activate human complement, see, e.g., the right column, lines 27-28, of page 1399 of Thomas et al., 1996, *Id.,* and the bridging paragraphs of pages 446 to 448 and 450 to 451 of Mueller et al, 1997, *Id.* Thus, Applicant submits that it was well-known to one of ordinary skill in the art at the time of filing of priority applications that eculizumab has a G2/G4 Fc portion, i.e., a mutated Fe portion.

Id. at 838–839 (bolding added). Alexion's statement, above, supports both Thomas's disclosure of eculizumab in the IgG4 isotype as the first construction of eculizumab, as well as an understanding that "eculizumab" referred to and refers to a genus of antibodies, including one with the hybrid IgG2/IgG4 isotype of the challenged claims. Accordingly, in view of the record before us, we conclude that "eculizumab" referred to and refers to a class or category of anti-C5 antibodies, also called 5G1.1 or h5G1.1 mAbs.

Upon considering the facts here in view of Petitioner's reliance on *Crish*, 393 F.3d 1253, and Patent Owner's reliance on *Endo Pharms.*, 894 F.3d 1374, we find the latter case analogous here. In *Crish*, the Federal Circuit found that a claim to the nucleotide sequence of the hINV gene promoter region was inherently disclosed by prior art that "specifically identified [the promoter region] by size and location" because "[t]he starting material plasmid necessarily contain[ed] the gene of interest, including the promoter region." *Crish*, 393 F.3d at 1257–59. Here, however, Hillmen does not "specifically identify" an eculizumab antibody containing the hybrid IgG2/IgG4 sequence of SEQ ID NO: 2, as required by claim 2.

Endo Pharms. compels a different result under the facts here. In *Endo Pharms.*, the issue was again inherency (in the context of obviousness) where prior art scientific articles described clinical trials for a testosterone undecanoate composition, but did not disclose its specific, and later claimed, formulation including a particular mixture of castor oil and benzyl benzoate. *Endo Pharms.*, 894 F.3d at 1278. It was later confirmed that the composition of the clinical trials described in the prior art articles did, indeed, have the claimed mixture of castor oil and benzyl benzoate. *Id.* The Federal Circuit held that because it was not demonstrated that a skilled artisan could extrapolate the vehicle formulation (a mixture of castor oil and benzyl benzoate) used in the prior art articles based on reported pharmacokinetic performance data, such performance could not have only been attributed to the claimed formulation and the generic disclosure of the pharmaceutical formulation in the prior art did not inherently disclose the claimed formulation. *Id.* at 1281–83. Furthermore, *Endo Pharms.*

distinguished *Crish* because it was about the inherent properties of a known prior art product, rather than a product that was named, but not known or determinable based on the prior art disclosure of its performance characteristics. *Id.* at 1383.

In Ground 1, the asserted prior art reference, Hillmen, discloses a clinical trial of "eculizumab," but does not otherwise identify the structure of the antibody tested, other than by referencing Thomas. Ex. 1004, 552, 553 (citing Ex. 1023). Thomas discloses a version of eculizumab different from that claimed (an IgG4 isotype rather than a hybrid IgG2/IgG4 antibody). Even accepting that a skilled artisan would have known of a hybrid IgG2/IgG4 antibody, as claimed (*see, e.g.*, Ex. 1002 ¶ 13), Hillmen's mere reference to "eculizumab" would have at least invoked a molecule having the IgG4 isotype taught in Thomas. Thus, Hillmen's disclosure of "eculizumab" would not have necessarily led the skilled artisan to the claimed antibody with "a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4." Moreover, none of the record references teaching or suggesting an IgG2/IgG4 isotype (e.g., Bowdish, Evans, Tacken, Mueller, or Mueller II) is cited in Hillmen. *See* Ex. 1004, 559. We disagree with Petitioner's position that such references' teaching would have somehow overridden Hillmen's direct invocation of Thomas's disclosure of eculizumab so as to point the skilled artisan to some alternative antibody structure.[18] Accordingly, under *Endo Pharms.*, as discussed above, there is no inherent anticipation of claim 2 by Hillmen.

---

[18] We need not address here Patent Owner's non-trivial argument that

To summarize, based on the preliminary record before us, as of the '880 patent's priority date, one of ordinary skill understood that eculizumab encompassed molecules of both IgG4 and IgG2/IgG4 isotypes, and a plain reading of Hillmen suggests that it specifies the former. Accordingly, one of ordinary skill in the art would not have necessarily understood Hillmen to disclose a version of eculizumab having the IgG2/IgG4 heavy chain constant region of SEQ ID NO: 2. For the above reasons, Petitioner has not demonstrated a reasonable likelihood that claim 2 of the '880 patent, is unpatentable under Ground 1.

E. Anticipation by Hill '05 (Ground 2)

In Ground 2, Petitioner challenges claim 2 as anticipated by Hillmen. Pet. 31–36. Patent Owner opposes. Prelim. Resp. 44–51.

1. The Parties' Contentions

In section I.F(2), above, we noted that Hill '05 reports on an extension of the clinical trial disclosed in Hillmen (discussed in part (1) of this same section). Petitioner's Ground 2 is, similarly, an extension of Ground 1 and relies on substantially the same or similar facts and bases in law. *See* Pet. 31–36. Petitioner argues "[a]s in Ground 1, the law also compels finding anticipation in Ground 2." *Id.* at 35 (again citing, *inter alia*, *Crish*, 393 F.3d at 1258).

Petitioner "has improperly attempted to shoehorn" the obviousness arguments of Grounds 5 and 6 into its anticipation contentions. Prelim. Resp. 45–46.

Petitioner contends, "Hill '05 is an Alexion publication describing results from a one-year extension study involving the same 11 patients enrolled in the Hillmen Phase 2 Pilot Study." Pet. 33 (citing Ex. 1047, 2559–60). Petitioner argues Hill '05, like Hillmen, is directed to and discloses administering "eculizumab" to such patients. *Id.* (citing Ex. 1047, 2565). Petitioner's rationale for Hill '05's anticipation of claim 2 is essentially the same as discussed above for Hillmen, only replacing Hillmen's generic disclosure for "eculizumab" with Hill '05's generic disclosure for "eculizumab," paired with the same identified admissions by Patent Owner during prosecution and the same contentions that Bowdish, Evans, and Mueller, rather than Thomas, would be the skilled artisan's basis for the structure of eculizumab. *See* Pet. 31–35 (citing Ex. 1015, 736, 783; Ex. 1047, abstract, 2559–60; Ex. 1002 ¶¶ 82–92; Ex. 1004, abstract, 554; Ex. 1042, Abstract; Ex. 1011, abstract; Ex. 1005 ¶¶ 81–96; Ex. 1012, abstract, 1235; Ex. 1013, abstract; Ex. 1006 ¶¶ 191–193, Figs. 13A, 13B; Ex. 1007, 44:4–13; Ex. 1008, 52–53, 58–61).

Patent Owner argued Grounds 1 and 2 together and, thus, makes essentially the same arguments over Ground 2 as discussed above regarding Ground 1. *See* Prelim. Resp. 44–51.

2. Analysis

At this stage in the proceedings and for the reasons discussed in section II.D above and as set forth below, we find Petitioner has not carried its burden to show a reasonable likelihood of anticipation of claim 2 under Ground 2.

Upon reviewing Hill '05, we find its disclosure to be no more specific or enlightening as to the structure of eculizumab than that of Hillmen. *See generally* Ex. 1047. Hill '05 states, "[w]e previously reported the outcome of an open-label study of eculizumab in patients with PNH.[2]" Ex. 1047, 2559 (reference "2" is Hillmen (Ex. 1004)). Hill '05 further states, "[e]culizumab is a humanized monoclonal antibody that specifically targets the complement protein C5 and prevents its cleavage.[9]" *Id.* (reference "9" is Thomas (Ex. 1023)). Thus, Hill '05, like Hillmen, invokes Thomas as a reference for eculizumab.

For the same reasons discussed above regarding Ground 1, based on the evidence presented at this stage in the proceedings, Petitioner has not shown that there is reasonable likelihood that claim 2 of the '880 patent is anticipated by Hill '05 under Ground 2.

F. Obviousness in view of Hillmen or Hill '05, combined with Bell and Wang (Grounds 3 and 4)

In Grounds 3 and 4, Petitioner challenges claims 1 and 3 as obvious in view of the combination of Hillmen, Bell, and Wang, and the combination of Hill '05, Bell, and Wang, respectively. Pet. 36–46. Patent Owner opposes. Prelim. Resp. 50–53. Pertinent to each of Grounds 3 and 4, as well as Grounds 5 and6, we address Patent Owner's secondary considerations evidence in section II(I), below.

1. The Parties' Contentions

For Ground 3, Petitioner states: "Bell disclosed a Phase 2 Pilot Study involving 11 PNH patients treated with eculizumab over a period of 12 weeks—identical to that disclosed in Hillmen. . . . Given the substantial

overlap, a POSA would have had ample reason to combine Hillmen and Bell, with a reasonable expectation of success at achieving the claimed subject matter." Pet. 36–37 (citations omitted). With respect to Ground 4, which substitutes Hill '05 for Hillmen, Petitioner again applies essentially "the same reasons discussed above in Ground 3. *See* Pet 44–46; Ex. 1002 ¶¶ 115–120.

Responding to these contentions, Patent Owner notes that, "Grounds 3 and 4 rely upon Hillmen or Hill, respectively, as the sole prior art allegedly disclosing the claimed element of an antibody comprising "a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4" in claims 1 and 3 of the '880 patent," but "neither Hillmen nor Hill disclosed the claimed antibody sequence either expressly or inherently." Prelim. Resp. 51. Patent Owner's point is well taken.

2. Analysis

As discussed in Sections II(D)(2) and II(E)(2) above, Petitioner has not established that Hillmen or Hill '05 inherently disclose the IgG2/IgG4 heavy chain constant region of SEQ ID No. 2, and the Petition sets forth no additional argument on that subject in support of Grounds 3 and 4. Petitioner also does not argue, nor do we discern, that the required sequence is taught in Bell or Wang. Accordingly, Petitioner has not demonstrated a reasonable likelihood that claims 1 and 3 are unpatentable over the combinations of Hillmen, Hill '0-5, Bell, and Wang set forth in Grounds 3 and 4.

G. Obviousness in view of Bell, Bowdish, Evans, and Wang (Ground 5)

In Ground 5, Petitioner challenges claims 1–3 as obvious over the combination of Bell, Bowdish, Evans, and Wang. Pet. 46–58. Patent Owner opposes. Prelim. Resp. 54–58. For the purpose of determining whether or not to institute inter partes review, we focus on claim 2.[19]

1. The Parties' Contentions

With respect to Ground 5, Petitioner contends that Bell and Wang taught all limitations of the challenged claims but for eculizumab's amino acid sequence. Pet. 45–46. Petitioner argues that one of ordinary skill in the art would have looked to Bowdish and Evans to obtain the amino acid sequence of eculizumab. *See* Pet. 580–55.

Petitioner contends that the skilled artisan would have looked to Bowdish for the light chain and most of the heavy chain sequences of eculizumab in light of Bowdish's use of an "anti-C5 antibody as the starter 'scaffold' antibody sequence for creating a recombinant TPO-mimetic" antibody. Pet. 50; Ex. 1002 ¶¶ 140–145. According to Petitioner, "Bowdish provided the full antibody amino acid sequence except for the heavy chain CDR3 (HCDR3) sequence, which was replaced with the TPO-mimetic peptide sequence (LPIEGPTLRQWLAARAPV)." *Id.* (citing Ex. 1006 ¶¶

[19] In addition to challenging Petitioner's positions with respect to claim 2, Patent Owner also raises non-trivial arguments in response to Petitioner's arguments regarding claims 1 and 3, which we need not address at this stage of the proceeding. *See* Pet. 41–50, 58–59; Prelim. Resp. 5–6, 42–43, 53, 58, 60.

191–193, Figs. 13A (SEQ ID NO: 67; "5G1.1 – TPO Heavy Chain (Bold Denotes TPO mimetic) Amino acid sequence"), 13B (SEQ ID NO: 69; "5G1.1 Light Chain Amino Acid Sequence"); Ex. 1002 ¶¶ 140–145). In particular, Petitioner argues:

> "A POSA . . .would have understood that the only portion of the only portion of the heavy chain sequence missing from Bowdish is the HCDR3 sequence because Bowdish taught that "[t]he TPO mimetic peptide graft in Fab clone X4b has been *transplanted into the heavy chain CDR3* of another antibody framework, *5G1.1* . . . The sequence was *cloned into 5G1.1* in such a fashion as to *replace the native CDR3*."

Pet. 50 (citing Ex. 1006 ¶ 191; Ex. 1002 ¶ 144–150 (emphasis Petitioner's)).

Petitioner further argues that to obtain the CDR3 sequence missing from Bowdish, one of ordinary skill in the art would have looked to Evans "because both Bell and Bowdish explicitly direct the artisan there for information on how the h5G1.1 antibody was originally created." *Id.* at 53 (citing Ex. 1002 ¶ 142; Ex. 1006 ¶ 191. Alternatively, Petitioner argues, one of ordinary skill in the art would have readily identified the entire IgG2/IgG4 heavy chain sequence from Bowdish alone, because Bowdish and Bell expressly cite and "incorporate[] by reference Evans for making eculizumab." *Id.* at 5 & n.2, 15, 53; Ex. 1006 ¶ 191; Ex. 1005 ¶ 52; Ex. 1002 ¶¶ 145–147.

According to Petitioner, "a POSA would have understood that *each* of the 5G1.1 antibody heavy chain variable regions in Evans contain *the same CDR3 sequence*: YFFGSSPNWYFDV. Thus, regardless of which 'version' of Evans' humanized 5G1.1 the POSA selected to combine with Bowdish, that heavy chain would contain the YFFGSSPNWYFDV CDR3 sequence."

Id. at 54 (internal citations omitted) (*citing e.g.*, Ex. 1002 ¶ 146). In sum, the "prior art would have led a POSA to make a simple substitution of one known element for another—i.e., replace the TPO-mimetic peptide sequence in Bowdish's antibody with the HCDR3 sequence from Evans—to yield predictable results: a humanized anti-C5 antibody." Pet. 56–57.

Petitioner relies on the Balthasar Declaration as support and further explanation as to how one of ordinary skill in the art would derive the claimed antibody based on the asserted art. *See* Pet. 50–58. Dr. Balthasar provides a number of illustrations useful to understanding Petitioner's argument.

Figure 4 from Dr. Balthasar's report, relating to the eculizumab light chain sequence, is reproduced below.

```
Bowdish SEQ ID NO. 69:    23 DIQMTQSPSSLSASVGDRVTITCGASENIYGALNWYQQKPGKAPKLLIYGATNLADGVPS 82
                             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
'880 SEQ ID NO. 4:         1 DIQMTQSPSSLSASVGDRVTITCGASENIYGALNWYQQKPGKAPKLLIYGATNLADGVPS 60

Bowdish SEQ ID NO. 69:    83 RFSGSGSGTDFTLTISSLQPEDFATYYCQNVLNTPLTFGQGTKVEIKRTVAAPSVFIFPP 142
                             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
'880 SEQ ID NO. 4:        61 RFSGSGSGTDFTLTISSLQPEDFATYYCQNVLNTPLTFGQGTKVEIKRTVAAPSVFIFPP 120

Bowdish SEQ ID NO. 69:   143 SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT 202
                             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
'880 SEQ ID NO. 4:        121 SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT 180

Bowdish SEQ ID NO. 69:   203 LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC 236
                             |||||||||||||||||||||||||||||||||
'880 SEQ ID NO. 4:        181 LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC 214
```

Figure 4: Comparison of the '880 patent's SEQ ID NO: 4 with Bowdish's SEQ ID NO: 69.

Ex. 1002 ¶ 53. Figure 4 purports to show identity between the mature peptide sequence[20] of the eculizumab light chain disclosed in Bowdish and

[20] Dr. Balthasar notes that Bowdish's Figures 13A and 13B identify leader sequences, not included in the comparisons of Figures 4 and 5 because one

SEQ ID NO: 4 of the '880 patent. *Id.*

Figure 5 from Dr. Balthasar's report, relating to the heavy chain sequence of eculizumab, is reproduced below.

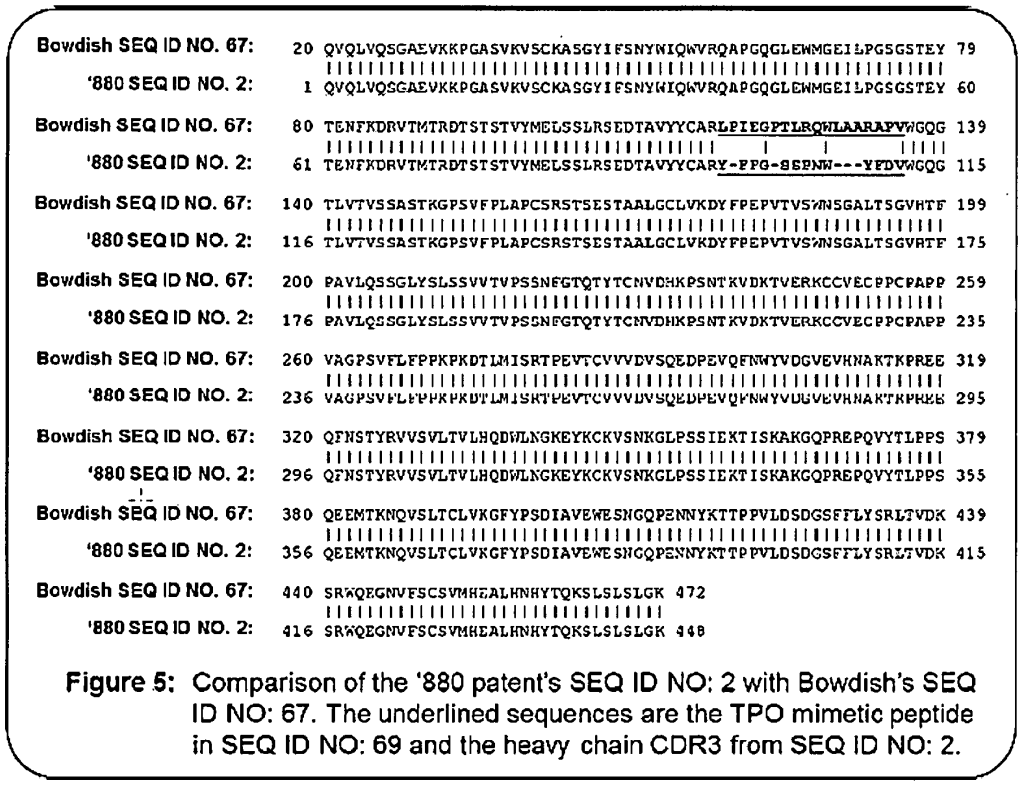


Figure 5: Comparison of the '880 patent's SEQ ID NO: 2 with Bowdish's SEQ ID NO: 67. The underlined sequences are the TPO mimetic peptide in SEQ ID NO: 69 and the heavy chain CDR3 from SEQ ID NO: 2.

Id. According to Dr. Balthasar, "Figure 5 shows that the mature portion of SEQ ID NO: 67 from Bowdish (*i.e.*, amino acids 20 to 472) aligns perfectly with SEQ ID NO: 2 from the '880 patent outside of the heavy chain CDR3 region." *Id.*

---

of ordinary skill in the art "would have understood that leader sequences are cleaved off as a standard part of the maturation of an antibody." *Id.*

Figure 6 of Dr. Balthasar's declaration, relating to the heavy chain CDRs of eculizumab, is reproduced below.

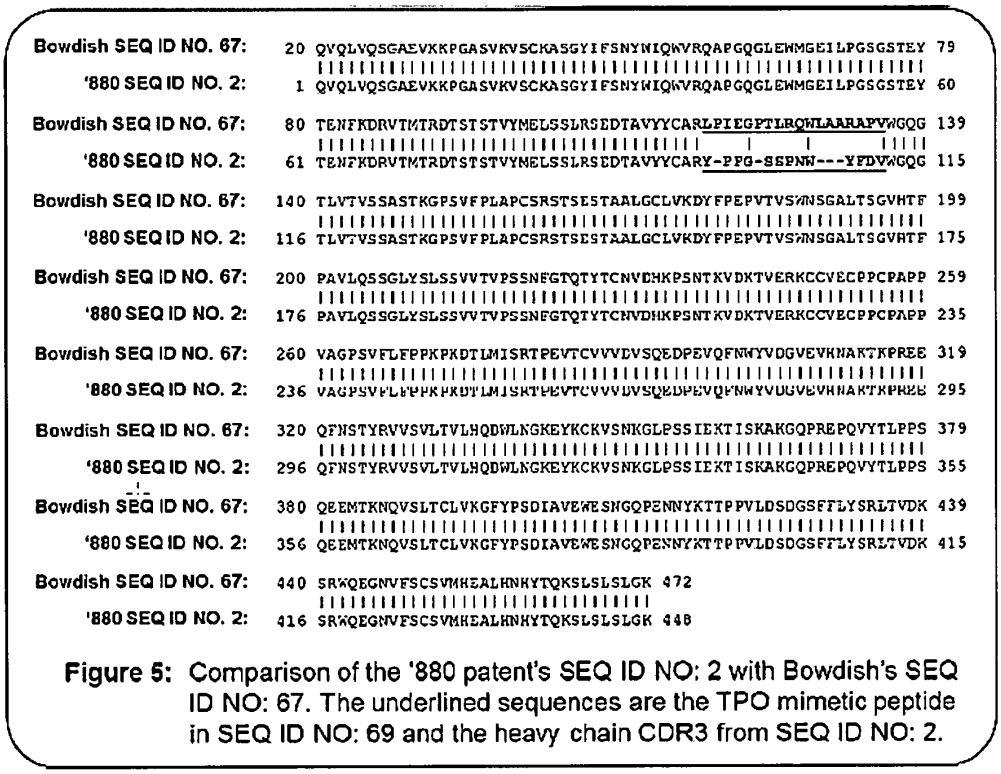


Figure 5: Comparison of the '880 patent's SEQ ID NO: 2 with Bowdish's SEQ ID NO: 67. The underlined sequences are the TPO mimetic peptide in SEQ ID NO: 69 and the heavy chain CDR3 from SEQ ID NO: 2.

Ex. 1002 ¶ 55. According to Dr. Balthasar, Figure 6 shows an alignment of SEQ ID NO: 2 of the '880 patent with the heavy chain CDRs identified in Evans underlined, including the YFFGSSPNWYFDV sequence of CDR3. *Id.* ¶¶ 55, 143.

Reproduced below is Figure 11 of Dr. Balthasar's declaration, relating to the relationship between the heavy chain CDR3 and Bowdish's TPO peptide.

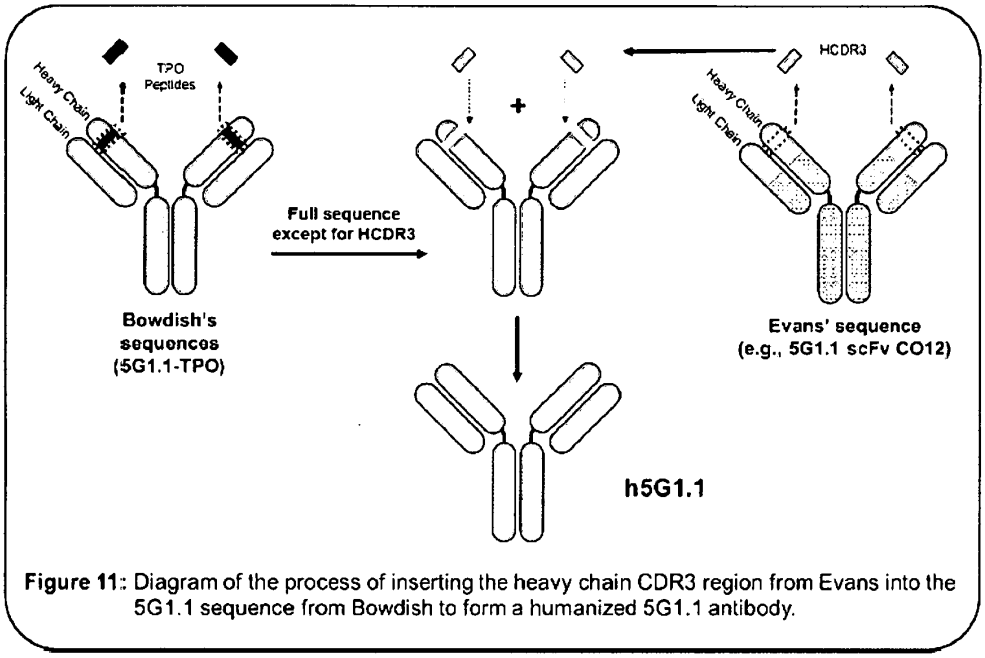


Figure 11: Diagram of the process of inserting the heavy chain CDR3 region from Evans into the 5G1.1 sequence from Bowdish to form a humanized 5G1.1 antibody.

Ex. 1002 ¶ 122. Figure 11 shows the original Bowdish scaffold antibody, including the location of eculizumab heavy chain CDR3; the use of Evans as a source of the HCDR3; and the replacement of Bowdish's TPO peptide with Evan's HCDR3. *Id.* According to Dr. Balthazar, Figure 11 "illustrate[s] how a POSA would have arrived at the claimed sequence (*i.e.*, eculizumab) by placing the heavy chain CDR3 disclosed in Evans into the 5G1.1 antibody sequence disclosed in Bowdish." *Id.*

With respect to reason to combine, Petitioner argues that, because the combination of Bell and Wang taught the use of eculizumab for the

treatment of PNH, wherein it was well known "that eculizumab was also known in the art as humanized '5G1.1,' 'h5G1.1,' or 'h5G1.1-mAb' with a hybrid IgG2/IgG4 constant region," the skilled artisan would have looked to e.g., Bowdish and Evans to recreate the molecule used in those successful studies. *See* Pet. 55; Ex. 1002 ¶¶ 147–151. Moreover, with respect to the specific sequence of SEQ ID NO: 2, Petitioner asserts that, as of the filing date of the '880 patent, it was well known "that antibodies with a hybrid IgG2/IgG4 constant region carried certain benefits, such as a reduced ability to elicit unwanted inflammatory events and lessened propensity to activate the complement system." Pet. 14 (citing Ex. 1032, 11, 19, 28; Ex. 1031, 451; Ex. 1002 ¶¶ 48, 56); *see* Ex. 1002 ¶¶ 24, 166. As noted by Dr. Balthasar, for example:

> A POSA would have been aware that "the HuG2/G4 antibody design should prove useful in humanization of other antibodies intended for human use where elimination of FcR binding and C [*i.e.*, complement] activation may be desirable." AMG1031, 451; *see also*, AMG1034, 1280. Because the goal of using eculizumab in treating PNH is to reduce the level of complement activation, using a heavy chain constant region that avoids a counter-productive activation of complement by the therapeutic antibody aligns well with the desired outcome. AMG1005, ¶¶[0003], [0012].

Ex. 1002 ¶ 48.

Patent Owner argues that, absent impermissible hindsight, the skilled artisan would not have combined, or reasonably expected success in combining the asserted references. Prelim. Resp. 4–5, 57. Patent Owner argues that Bell, like Hillmen and Hill '05, taught eculizumab was the antibody of Thomas and nothing in the other references would point the

skilled artisan toward a different antibody, e.g., the IgG2/IgG4 hybrid covered by the claim. *Id.* Patent Owner also argues Thomas taught away from the claimed invention because Thomas described an "eculizumab" with an IgG4 constant region. *Id.* at 55; *see also id.* at 51–52 (arguing that Bell, Hillmen, and Hill '05 taught away from the IgG2/IgG4 version of eculizumab).

Patent Owner argues that even assuming one of ordinary skill did combine the cited references to obtain eculizumab having the claimed sequence, "a POSA would not have reasonably expected the resulting compound to work in a pharmaceutical composition for preventing C5 cleavage and safely and effectively treating PNH . . . . [because] even small changes to the amino acid sequence could have a substantial impact on the binding properties and the safety and efficacy of an antibody intended for human administration." *Id.* at 57–58 (citing *Novartis Pharm. Corp. v. West-Ward Pharms. Int'l Ltd.*, 923 F.3d 1051 (Fed. Cir. 2019). Patent Owner thus contends that, in view of this complexity and unpredictability, the skilled artisan would not have ventured away from Thomas's known antibody; i.e., would not have looked to Bowdish and Evans for an isotype variant of that antibody. *Id.* at 40–41.

2. Analysis

At this stage in the proceedings and for the reasons discussed below, we find Petitioner has carried its burden to show a reasonable likelihood that, at least, claim 2 of the '880 patent would have been obvious under Ground 5.

Regarding Patent Owner's argument that the combination of Bell, Bowdish, Evans, and Wang involves improper hindsight and that the combination fails to render the challenged claims obvious because Bell's disclosed antibody would necessarily be that of Thomas's disclosure, we are not convinced. As summarized in Section I(F)(3), above, Bell extols the virtues of eculizumab for the treatment of PNH, but does not identify its amino acid sequence. Bell does cite to and incorporate by reference Thomas, thus, Thomas's IgG4 isotype antibody would be one type of eculizumab contemplated by Bell. But, as discussed in section II(D)(2), one of ordinary skill in the art would have understood that "eculizumab" encompassed multiple isotypes, including one having the hybrid IgG2/IgG4 isotype noted in Tacken and Bowdish. Here, Bowdish discloses a substantial portion of the anti-C5 antibody 5G1.1 and points to Evans as evidencing the remaining amino acid sequence. And, with respect to Patent Owner's argument that neither Bowdish nor Evans uses the term "eculizumab" (Prelim. Resp. 58–59), on the present record, we accept from Dr. Balthasar's Declaration that one of ordinary skill in the art would have looked to those references for the structure of eculizumab, particularly given that Bowdish incorporates Evans for the construction of 5G1.1. *See, e.g.*, Ex. 1006 ¶ 191; Ex. 1002 ¶¶ 54, 78, 79, 89, 90, 140–148, 151. Accordingly, Petitioner's arguments rely on the disclosures of the prior art and no improper hindsight is necessarily invoked under Petitioner's rationale.

As for Patent Owner's contention that Thomas taught away from the claimed invention (or, somehow taught away from the prior art combination), we disagree. A "teaching away" requires a reference to

actually criticize, discredit, or otherwise discourage the claimed solution. *See In re Fulton*, 391 F.3d 1195, 1201 (Fed. Cir. 2004). "[T]he question is whether there is something in the prior art as a whole to suggest the desirability, and thus the obviousness, of making the combination, not whether there is something in the prior art as a whole to suggest that the combination is the most desirable combination available." *Id.* at 1200 (citation and emphasis omitted). "The prior art's mere disclosure of more than one alternative does not constitute a teaching away from . . . alternatives because such disclosure does not criticize, discredit, or otherwise discourage the solution claimed." *Id.* at 1201. Thomas does not criticize, discredit, or discourage the claimed invention or the prior art combination. Thomas, at most, teaches the original eculizumab construction, an alternative to the version as taught by Bowdish and Evans.

On the present record, we are also not persuaded by Patent Owner's argument that, "[e]ven if Bowdish and Evans were combined . . . , a POSA would not have reasonably expected the resulting compound to work in binding to C5 or safely and effectively treating PNH" because even small changes could substantially impact an antibody's binding properties, safety, and efficacy for human administration. *See* Prelim. Resp. 57.

As an initial matter, claims 2 and 3 are not directed to the treatment of any particular disease. And, although claim 1 recites "[a] pharmaceutical composition for use in treating a patient afflicted with paroxysmal nocturnal hemoglobinuria (PNH)," it is not clear whether claim 1 requires any showing of safety and efficacy as these are standards for FDA approval of new drugs rather than of patent law. *Helsinn Healthcare S.A. v. Teva*

Pharms. USA, Inc., 855 F.3d 1356, 1372–73 (Fed. Cir. 2017), *cert. granted*, 138 S. Ct. 2678 (2018), and *aff'd*, 139 S. Ct. 628 (2019) ("Approval of a new drug by FDA, however, is a more demanding standard than that involved in the patents-in-suit. The patents here make no reference to FDA standards and broadly claim a palonosetron formulation for reducing the likelihood of emesis and CINV.").

Further, Bell teaches that a variety of compounds containing the variable regions of a humanized 5G1.1 are useful to the treatment of PNH, stating: "In particularly useful embodiments, the compound is an anti-C5 antibody selected from the group consisting of h5G1.1-mAb (eculizumab), h5G1.1-scFv (pexelizumab) and other functional fragments of h5Gl.l." Ex. 1005 ¶ 12. Moreover, Bell touts the benefits of "eculizumab" in a long term clinical trial, indicating that the treatment is suitable "for use in treating a patient afflicted with paroxysmal nocturnal hemoglobinuria (PNH)," as set forth in claim 1. As noted above, one of ordinary skill in the art understood eculizumab to encompass a humanized 5G1.1 antibody having the IgG2/IgG4 isotype indicated in SEQ ID NO: 2.

Although we recognize Patent Owner's argument that "sequence changes outside of the antigen-binding site, *e.g.*, in the heavy chain constant region" may influence antibody affinity, specificity, and immunogenicity, this is counterbalanced by Petitioner's citation to eculizumab of the IgG2/IgG4 isotype and its reasoned argument that the skilled artisan would look to the hybrid sequence because it was known "that antibodies with a hybrid IgG2/IgG4 constant region carried certain benefits, such as a reduced ability to elicit unwanted inflammatory events and lessened propensity to

activate the complement system." *See* Prelim. Resp. 11; Pet. 14 (citing Ex. 1032, 11, 19, 28; Ex. 1031, 451; Ex. 1002 ¶¶ 48, 56).

On balance, the present record suggests that a person of ordinary skill in the art would have reasonably expected a version of eculizumab having a heavy chain consisting of SEQ ID NO: 2 to comprise and anti C5 antibody as required in claims 2 and 3, and to work for the purpose set forth in claim 1 ("treating a patient afflicted with paroxysmal nocturnal hemoglobinuria (PNH)"). The parties are invited to further address these points during trial.

We take note of Patent Owner's arguments relating to, and evidence of, objective indicia of non-obviousness, discussed in Section II(I), below. Although we note that there is some evidence to support Petitioner's contentions of commercial success, long-felt but unmet need, and industry praise, we also note some potential shortcomings in Patent Owner's presentation, including the rebuttability of the presumption of a nexus between their evidence and the claimed and novel subject matter. We expect this issue will be further developed at trial.

Again, to summarize, based on the evidence presented at this stage in the proceedings, it has been shown that there is reasonable likelihood that at least claim 2 of the '880 patent would have been obvious over Bell, Bowdish, and Evans under Ground 5.

H. Obviousness in view of Bell, Evans, Mueller, and Wang (Ground 6)

In Ground 6, Petitioner challenges claims 1–3 as obvious in view of Bell, Evans, Mueller, and Wang. Pet. 58–68. Patent Owner opposes. Prelim. Resp. 58–62; *see also id.* at 4–5, 32–39.

1. The Parties' Contentions

Referencing its arguments for Ground 4, Petitioner contends that, "Bell and Wang together taught all of the elements of claims 1–3 except for the claimed antibody sequences.'" Pet. 58. Petitioner then points to Evans and Mueller as teaching the amino acids of SEQ ID NOs: 2 and 4. *Id.* at 59–66. As stated by Petitioner in the context of Ground 1: "**Evans** disclosed the amino acid sequences of eculizumab's heavy and light chain variable domains, and **Mueller** disclosed the hybrid IgG2/IgG4 heavy chain and light chain constant domains of eculizumab." *Id.* at 28 (citing Ex. 1007, 44:4–13; Ex. 1008, 52–53, 58–61; Ex. 1002 ¶78); *see id.* at 59–66 (citing, e.g., Ex. 1002, ¶¶ 156, 165, 166, 170–175). Petitioner further argues:

> Knowing that chimeric IgG2/IgG4 constant regions that were known not to activate the complement system (AMG1008, 7:28-31, 8:23-26, 12:27-32), a POSA reading Bell and Evans also would have looked to Mueller for "h5G1.1" sequence information. AMG1002, ¶¶166, 173. Mueller taught methods for making "chimeric antibodies containing the C1 and hinge region of human IgG2 and the C2 and C3 regions of human IgG4 … (HuG2/G4 mAb)." AMG1008, 12:27-30; *see also, id.*, 8:23-26. In particular, Mueller described a control antibody "h5G1.1 CO12 HuG2/G4 mAb," which a POSA would have readily identified as a humanized anti-C5 antibody because of the "h5G1.1" nomenclature coupled with the hybrid IgG2/IgG4 constant region ("HuG2/G4"). AMG1008, 12:37, FIG. 15; AMG1005, ¶[0052]; AMG1034, 1279; AMG1049, 838-839; AMG1002, ¶166, 173–174.

Id. at 61–62.

Figure 14 from the Balthasar Declaration is reproduced below.

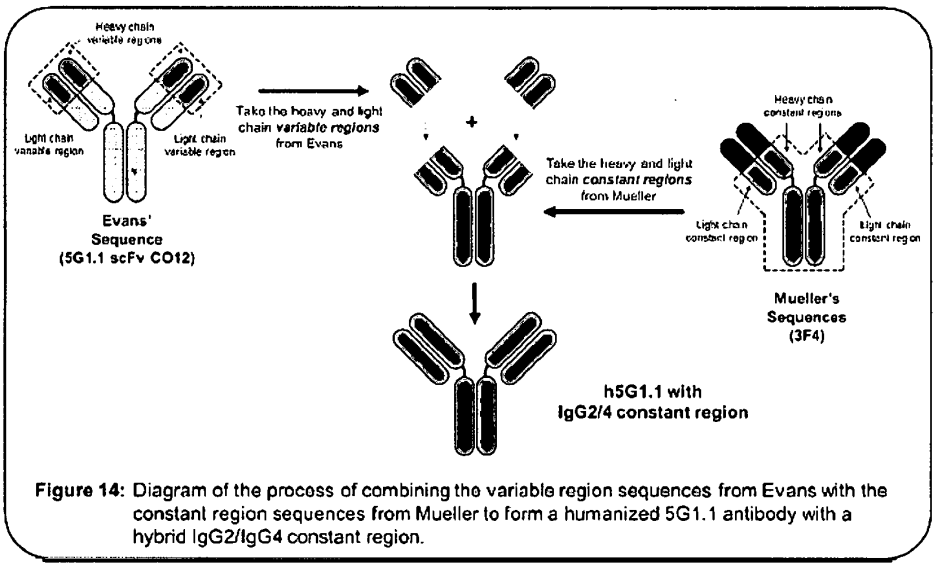


Figure 14: Diagram of the process of combining the variable region sequences from Evans with the constant region sequences from Mueller to form a humanized 5G1.1 antibody with a hybrid IgG2/IgG4 constant region.

Ex. 1002 ¶ 156. According to Petitioner, the above figure presents an overview of how one of ordinary skill in the art would have combined the teachings of Evans and Mueller to arrive at the complete eculizumab antibody sequence described in claim 1. *See* Pet. 60.

Relying on Dr. Balthasar's testimony, Petitioner argues that "Mueller disclosed the amino acid sequence of a hybrid IgG2/IgG4 heavy chain constant domain when Mueller disclosed the sequence of the chimeric anti-VCAM '3F4' antibody." Pet. 64 (citing Ex. 1002 ¶ 169, 173; Ex. 1008, 58–61). "Given that Mueller used this humanized anti-C5 antibody as an isotype control for 3F4 HuG2/G4, a POSA would have reasonably expected the disclosed heavy and light chain constant regions to be the same as those in the 3F4 HuG2/G4 chimeric antibody. AMG1002, ¶¶57, 166." *Id.* at 63. Accordingly Petitioner argues, one of ordinary skill in the art would have

aligned Mueller's chimeric 3F4 HuG2/G4 heavy chain and mature 3F4 heavy and light chain variable regions such that a skilled artisan aligning the two would identify the 3F4 variable regions (at Figure 9) as amino acids 20–137 of the 3F4 HuG2/G4 heavy chain and amino acids 20–131 of the 3F4 light chain. *Id.* at 62 (citing e.g., Ex. 1002 ¶ 173). Petitioner contends that the skilled artisan "would have immediately known that the remainder of the 3F4 HuG2/G4 heavy chain (amino acids 138-463 is the hybrid IgG2/IgG4 constant region of that antibody, and that the remainder of the 3F4 chain (amino acids 132–238) is the light chain constant region of that antibody." *Id.* at 63 (citations omitted). With this understanding of the heavy and light chain constant domain sequences in mind, Petitioner contends the skilled artisan would have looked to Evans to complete the whole antibody using Evans's variable regions identified from its SEQ ID NO: 20, particularly because Evans uses the same "CO12" nomenclature to refer to its 5G1.1 scFv as Mueller does in referring to h5G1.1. *Id.* at 63–65 (citing Ex. 1002 ¶¶ 156, 167–168, 172).

Petitioner's rationale for combining Mueller and Evans is that Mueller taught antibodies with lower immune response and identified an antibody as h5G1.1 CO12 HuG2/G4 mAb, which the skilled artisan would have known is eculizumab. Further, Petitioner argues, Evans taught the complementary parts of this anti-C5 antibody, so, by combining the elements of the two references, a complete antibody would have been created having the SEQ ID NO: 2 and SEQ ID NO: 4 of the claim. *Id.* at 66–67; *see also id.* at 67–68 (further discussing why one of ordinary skill in the art would have combined the cited references with a reasonable expectation of success).

Patent Owner argues "[a] POSA would have understood that Mueller could have used ***any*** antibody with an IgG4 or IgG2/G4 isotype as a 'negative control' for its *in vitro* experiments, as long as it did not bind to VCAM," meaning, there would be no reason to incorporate the variable regions taught by Evans. *Id.* at 59. And, according to Patent Owner, without improper hindsight, the skilled artisan would not have sought to combine Mueller and Evans. *See, e.g.*, Prelim. Resp. 3–4. Patent Owner argues "[a] POSA as of March 15, 2007 considering the problem addressed by the '880 patent – designing anti-C5 antibody compositions to prevent cleavage of C5 and to treat PNH – would have had no reason to look at Mueller, which had nothing to do with that problem. *Id.* at 59. (citing *Broadcom Corp. v. Emulex Corp.*, 732 F.3d 1325, 1334 (Fed. Cir. 2013) ("While a prior art reference may support any finding apparent to a person of ordinary skill in the art, prior art references that address different problems may not, depending on the art and circumstances, support an inference that the skilled artisan would consult both of them simultaneously.").

2. Analysis

In contrast to Ground 5, where Bowdish expressly incorporates Evans, thereby rendering the combination unquestionable, the references asserted in Ground 6 provide no express link between the teachings of Mueller and Evans. Although such a combination is possible, the mere fact that prior art can be combined does not establish that one of ordinary skill would have done so. *See, e.g.*, *In re Fritch*, 972 F.2d 1260, 1266 (Fed. Cir. 1992) (The "mere fact that the prior art may be modified in the manner suggested . . .

does not make the modification obvious unless the prior art suggested the desirability of the modification.").

Ground 6, thus, presents a close question on whether there would have been sufficient motivation to combine Mueller and Evans in the manner argued by Petitioner. Upon review of the Balthasar Declaration, it is apparent that Mueller's 3F4 heavy chain provides a match for part of the claimed SEQ ID NO: 2. *See* Ex. 1002 ¶ 58, Figure 8. Further, Mueller's 3F4 light chain provides a match for part of the claims SEQ ID NO: 4. *Id.* ¶ 59, Figure 9.

Figure 10 of the Balthasar Declaration, reproduced below, illustrates the extent that each of Evans and Mueller (and Bowdish, per Ground 5) discloses the claimed SEQ ID NOs: 2 and 4.

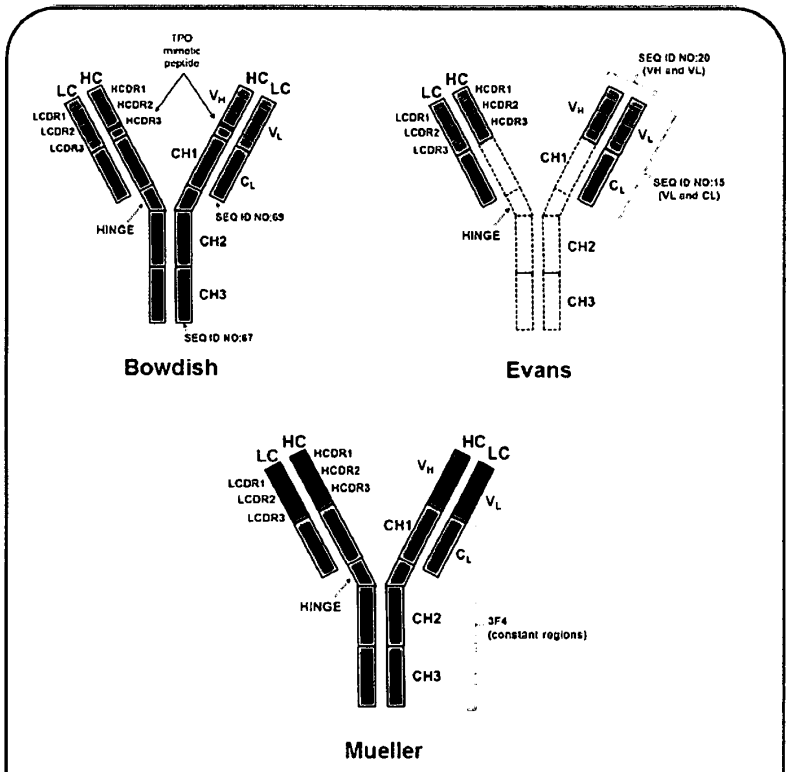


Figure 10: Diagram of the portions of the claimed antibody sequence disclosed in Bowdish (SEQ ID NOs: 67 and 69), Evans (SEQ ID NOs: 15 and 20), and Mueller (as part of the 3F4 antibody). *Green*: sequences matching SEQ ID NOs: 2 and 4 from the '880 patent; *blue*: other sequences

Ex. 1002 ¶ 60. Dr. Balthasar's Figure 10 shows three antibody structures: Bowdish top-left, Evans top-right, and Mueller bottom center. The Figure shows identity with SEQ ID NOs: 2 and 4 of the '880 patent in green and differences in blue.

Based on the above figure, Petitioner's rationale for combining the teachings of Mueller and Evans is somewhat tenuous. In particular, we are concerned with the reasons one of ordinary skill in the art would have paired Mueller with Evans with, choosing precisely those portions of Mueller's and Evan's constructs to create an antibody having exactly the sequences set forth SEQ ID NOs. 2 and 4. At this stage in the proceedings, based on the evidence before us, the answer is not entirely clear and Patent Owner's argument regarding improper hindsight makes some sense. True, Mueller discloses "a humanized antibody directed against human C5 (h5G1.1 CO12 HuG4 mAb)," but little else regarding its structure. *See* Ex. 1008, 12. It is not apparent that the skilled artisan, knowing of Evans, would have looked to Mueller, or vice versa.

Based on the evidence presented at this stage in the proceedings, Petitioner has not sufficiently demonstrated a reasonable likelihood that claims 1–3 of the '880 patent would have been obvious over the combination set forth in Ground 6.

I. Objective Evidence of Non-Obviousness

Factual considerations underlying the obviousness inquiry include the scope and content of the prior art, the differences between the prior art and the claimed invention, the level of ordinary skill in the art, and any relevant secondary considerations. *See Graham*, 383 U.S. at 17–18. Relevant secondary considerations include commercial success, long-felt but unsolved needs, failure of others, and unexpected results. *KSR*, 550 U.S. at 406, (2007). Although evidence pertaining to secondary considerations must be taken into account whenever present, it does not necessarily control the

obviousness conclusion. *See, e.g.*, *Pfizer, Inc. v. Apotex, Inc.*, 480 F.3d 1348, 1372 (Fed. Cir. 2007).

Petitioner contends no objective indicia of non-obviousness support patentability of the challenged claims. Pet. 38, 46, 58, 68–70. In particular, Petitioner notes that during the prosecution of the related '504 patent, Alexion argued that the claimed heavy chain of eculizumab, i.e., the hybrid IgG2/IgG4 constant domain, provided surprising and unpredictable results, such as decreased effector function, reduced immunogenicity, and increased half-life. Pet. 68–69 (citing Ex. 1014, 588, 593 (¶ 8)). However, Petitioner contends, because eculizumab's hybrid IgG2/IgG4 constant region was well known in the art (as evidenced by Tacken), the allegedly surprising and unpredictable features of the antibody have no nexus to the challenged claims. *Id.* at 69 (citing Ex. 1034, 1279).

Petitioner further contends that one of ordinary skill in the art would not have found surprising the alleged beneficial results of using the hybrid constant region of SEQ ID NO: 2 because, in view of Mueller II, antibodies with the claimed hybrid IgG2/IgG4 heavy chain were known not to bind FcR and to be less immunogenic, whereas it was well known that antibodies with this claimed hybrid heavy chain would have an increased half-life. *Id.* (citing Ex. 1031, 488, 451; Ex. 1032 ("Rother"), 5, 19[21]; Ex. 1002 ¶ 180–182); *see, e.g.*, Ex. 1032, 5–6 (disclosing that antibodies having "an engineered constant region that includes an IgG2-derived portion and an IgG4-derived portion . . . maintain the function of the non-Fc component

[21] Rother et al., WO 2005/007809 A2, published Jan. 27, 2005 (Ex. 1032).

and/or have increased half-life compared to the non-Fc component alone and/or lack unwanted antibody Fc-mediated cell activating and inflammatory properties including events resulting from Fc-receptor antibody engagement and complement activation").

Patent Owner contends that evidence of commercial success, long-felt, but unmet need, and industry praise support the non-obviousness of the challenged claim. Prelim. Resp. 61. Patent Owner argues that SOLIRIS, the product embodying the claimed antibody, is a commercial success, having produced annual net product sales in excess of $1 billion in 2018. *Id.* (citing Ex. 2018, 70). Patent Owner further contends that this commercial success "has a direct nexus to the patented features of the '880 patent, which claims pharmaceutical compositions of the uniquely-engineered, non-naturally occurring antibody responsible for the drug's clinical (and therefore commercial) success as a treatment for PNH, as well as the complement-mediated hemolytic condition aHUS." *Id.*

At this stage in the proceedings, based on the evidence presented by Patent Owner, it is apparent that SOLIRIS is a successful product. "[T]here is a presumption of nexus for objective considerations when the patentee shows that the asserted objective evidence is tied to a specific product and that product "is the invention disclosed and claimed in the patent." *WBIP, LLC v. Kohler Co.*, 829 F.3d 1317, 1329 (Fed. Cir. 2016). This "presumption of nexus is rebuttable: a patent challenger may respond by presenting evidence that shows the proffered objective evidence was 'due to extraneous factors other than the patented invention.'" *Id.* Here, the parties appear to agree that the claim of the '149 patent is directed to the

commercial product SOLIRIS. However, commercial success "is relevant in the obviousness context only if there is proof that the sales were a direct result of the unique characteristics of the claimed invention – as opposed to other economic and commercial factors unrelated to the quality of the patented subject matter." *In re Huang*, 100 F.3d 135, 140 (Fed. Cir. 1996).

Patent Owner argues that, because SOLIRIS is "the first FDA-approved treatment to reduce hemolysis in patents with PNH," there is evidence that the claimed antibody fulfilled a long-felt, unmet need in the market. Prelim. Resp. 61–62 (citing Ex. 2019, 1270).

At this stage in the proceeding, the available evidence supports that anti-C5 antibodies were considered potential therapeutic options for "many years" before 2007, and that Alexion's eculizumab product "is currently the only complement-specific antibody on the market" and is the "first and only approved therapy for PNH." Ex. 2019, 1270. Again, it may be presumed that there is a nexus between the claimed and novel elements of the SOLIRIS product and the meeting of the long-felt need. However, "[w]here the offered secondary consideration actually results from something other than what is both claimed and *novel* in the claim, there is no nexus to the merits of the claimed invention." *In re Kao*, 639 F.3d 1057, 1068 (Fed. Cir. 2011).

Patent Owner also contends "SOLIRIS® also received industry praise as the recipient of multiple Prix Galien awards (the industry's highest accolade . . . ." Prelim. Resp. 62 (citing Ex. 2020; Ex. 2021).

As with the other two contended bases for indicia of non-obviousness, while it is apparent there was high praise for the SOLIRIS product from the

relevant industry, there is a rebuttable presumption that this praise has a nexus with the claimed subject matter. *Cf. In re Kao*, 639 F.3d at 1068.

Given the early stage of these proceedings, we decline to accord much weight to Patent Owner's substantially untested evidence of objective indicia of non-obviousness. The parties will have the opportunity to further develop these facts during trial, and the Board will evaluate the fully-developed record at the close of the evidence.

J. The Board's Discretion to Deny Institution under 35 U.S.C. §§ 325(d) and 314(a)

1. The Parties' Positions

Patent Owner argues the "Petition should also be denied institution under 35 U.S.C. §§ 325(d) and 314(a), because Amgen's Grounds rely on the 'same or substantially the same prior art or arguments' previously presented to the PTO" "[i]n the course of patent prosecution leading to issuance of the '880 patent, as well as prosecution of related U.S. Patent Nos. 9,719,880 ('the '880 patent') and 9,732,149 ('the '149 patent'). Prelim. Resp. 25, 62–63; *see id.* at 63 (asserting that the Examiner considered Hillmen, Evans, and Wang; and further reviewed Bell ("cumulative of Bowdish"), and Mueller II ("cumulative of Mueller"). Patent Owner argues that in the course of prosecution leading to the issuance of the '880 patent, the Examiner:

- Expressly discussed Amgen's asserted references Hillmen 2004 (AMG1004), Evans (AMG1007), and Wang (AMG1028) as a basis for rejection, before ultimately finding the claims to be allowable over the art (*see, e.g.*, AMG1014 at 557-561, 623-628, 738-743);

- Considered Amgen's asserted reference Bell (AMG1005), which Alexion submitted to the PTO (*see, e.g.*, AMG1014 at 566);
- Considered U.S. Patent No. 7,482,435 (ALXN2016), which is the parent to and cumulative of Amgen's cited "Bowdish" application (AMG1006), disclosing the same information on which Amgen relies here (*see, e.g.*, AMG1016 at 154); and
- Considered the "Mueller II" article (AMG1031), which is cumulative of Amgen's asserted "Mueller" reference (AMG1008) because, as Amgen's declarant recognized, it "discloses the same antibodies" as Mueller. (*See, e.g.*, AMG1016 at 625; AMG1002 ¶ 119–110 & n.12.)

Prelim. Resp. 17–18.

Petitioner's position on this issue is that "[t]he arguments and evidence presented herein were not before the examiner during prosecution and, therefore, do not constitute 'the same or substantially the same prior art or arguments' under 35 U.S.C. §325(d).'" Pet. 24. In particular, Petitioner argues that

> the examiner rejected Alexion's claims as obvious over combinations of Hillmen, Evans, Wang and Thomas during prosecution. Thomas was the only reference relied upon for eculizumab sequence information. AMG1016, 596-602. And, the examiner allowed the '880 patent claims mistakenly believing—because of Alexion's mischaracterization of the art—that the sequence and structure of eculizumab were not already known. *Id.*, 762-763.
>
> Though Hillmen, Evans, and Wang were referenced by the examiner during prosecution, this Petition presents them in a different light, along with new references—Bell, Bowdish, and Mueller—which teach the IgG2/IgG4 constant domain missing from the art combination raised during prosecution.

> Bell and a parent application to Bowdish (US 2003/0049683 A1) was cited but not relied upon during prosecution, and Mueller was not cited at all. Thus, the art combinations here, which were not raised by the examiner during prosecution, provide the complete sequence of eculizumab, thereby teaching the very thing the examiner mistakenly concluded was missing from the prior art. Consequently, this Petition is not the same/substantially the same as or cumulative of any previous arguments and § 325(d) does not preclude instituting this Petition.

Id. at 24–25.

2. Analysis

Regarding the Board's discretion under 35 U.S.C. § 325(d), in *Becton, Dickinson & Co. v. B. Braun Melsungen AG*, the Board enumerated non-exhaustive factors to be considered in exercising discretion under 35 U.S.C. § 325(d) on whether to institute *inter partes* review. Case IPR2017-01586, slip op. at 17–18 (PTAB Dec. 15, 2017) (Paper 8) (precedential as to § III.C.5, first paragraph). The non-exhaustive *Becton* factors are:

> 1. the similarities and material differences between the asserted art and the prior art involved during examination;
>
> 2. the cumulative nature of the asserted art and the prior art evaluated during examination;
>
> 3. the extent to which the asserted art was evaluated during examination, including whether the prior art was the basis for rejection;
>
> 4. the extent of the overlap between the arguments made during examination and the manner in which Petitioner relies on the prior art or Patent Owner distinguishes the prior art;
>
> 5. whether Petitioner has pointed out sufficiently how the Examiner erred in its evaluation of the asserted prior art; and

> 6. the extent to which additional evidence and facts presented in the Petition warrant reconsideration of the prior art or arguments.

Id. (numbering added). The *Becton* factors are not dispositive, but are part of a balanced assessment of the relevant circumstances in a particular case and we do not simply default to a tally of each factor to determine whether or not an IPR should be instituted.

Here, Patent Owner has not clearly identified how its arguments fall under the above-noted factors, but has generally argued that the prior art before us now was considered by the prosecuting Examiner either directly or as being cumulative of references that were so considered, and has further argued that the unpatentability issues presented in the Petition are the same as those at issue before the Examiner. Upon review of this evidence, we note that the Examiner considered Hillmen in rejecting a claim for obviousness-type double patenting and for anticipation. *See* Ex. 1014, 482, 557–561. As discussed in section II(D)(2), above, at this stage in the proceedings, the anticipation Ground 1 over Hillmen, on its own, is not considered sufficient to institute IPR; therefore, Hillmen's consideration during prosecution is not determinative here. Rather, we focus on the Bell, Bowdish, Evans, and Wang—the references of Ground 5, upon which we base our institution decision.

As an initial matter, Alexion did address the substance of Evans during prosecution. *See* Ex. 1014, 588. But, according to Patent Owner, the Examiner "[c]onsidered" Bell and "U.S. Patent No. 7,482,435 (ALXN2016), which is the parent to and cumulative of Amgen's cited "Bowdish" application (AMG1006), disclosing the same information on which Amgen

relies here." Prelim. Resp. 18. Both of these citations, however, are to an Information Disclosure Statement signed by the Examiner. *See id.* (citing Ex. 1014, 565, 566). Patent Owner does not direct us to, nor do we discern, where either Bell or some version of Bowdish was substantively considered during prosecution. The Board has consistently declined exercising its discretion under Section 325(d) when the only fact a Patent Owner can point to is that a reference was disclosed to the Examiner during the prosecution. *See, e.g.*, *Amneal Pharms. LLC v. Alkermes Pharma Ireland Ltd.*, IPR2018-00943, slip op. at 40 (PTAB Nov. 7, 2018) (Paper 8) (declining to deny institution based on Section 325(d) where the reference was listed on the face of the patent, but Patent Owner provided no evidence "about the extent to which the Examiner evaluated" the reference during prosecution); *Digital Check Corp. d/b/a ST Imaging v. E-Imagedata Corp.*, IPR2017-00178, slip op. at 12–13 (PTAB Apr. 25, 2017) (Paper 6) (acknowledging that a prior art reference was cited in an IDS, but granting institution because there was no indication that the claims were rejected based on those references or that the Examiner substantively discussed those references during prosecution); *Fox Factory, Inc. v. SRAM, LLC*, IPR2016-01876, slip op. at 7–9 (PTAB Apr. 3, 2017) (Paper 8) (refusing to deny institution based on Section 325(d) for grounds based on a prior art reference that was simply cited in an IDS and not considered at any length); *Praxair Distribution, Inc. v. INO Therapeutics LLC*, IPR2015-00893, slip op. at 8 (PTAB Sept. 22, 2015) (Paper 14) (granting institution even though the references were previously cited in an IDS because patent owner failed to identify with specificity where the references were considered); *HyperBranch Medical Technology, Inc. v.*

Confluent Surgical, Inc., IPR2018-01099, slip op. at 17 (PTAB Nov. 27, 2018) (Paper 14) (instituting IPR because, *inter alia*, "[t]he Examiner does not appear to have considered the combined teachings of Spero and Haber during examination of the '021 patent.").

We also note that the Examiner cited Wang in much the same context as argued here by Petitioner. *See* Ex. 1004, 560–561 (Examiner asserting that based on Wang, "the artisan of ordinary skill would have had a reasonable expectation of success in making a 10 mg/ml solution of the antibody sterile, preservative free solution"). We do not discern, however, where Alexion addressed this issue on the merits, arguing instead that "Wang fails to teach any part of the sequence of eculizumab, let alone its unique heavy chain." *Id.* at 587.

Based on the evidence presented by Patent Owner, *Becton* factors 1–6 weigh in favor of not exercising our discretion not to institute here. Therefore, based on the evidence cited by Patent Owner and for the reasons above, we decline to exercise our discretion under section 325(d) to deny institution here.

Other than the heading of Section VI of the Preliminary Response and that section's first sentence invoking the statute, Patent Owner presents no arguments or evidence directed to the Board's discretion under 35 U.S.C. § 314(a). Therefore, we also decline to exercise our discretion under section 314(a) to deny institution.

II. CONCLUSION

On the record before us at this stage in the proceeding, Petitioner has demonstrated a reasonable likelihood of prevailing on Ground 5 in showing that claim 2 of the '880 patent is obvious over the combination of Bell, Bowdish, and Evans. Given this determination, we institute trial on all challenged claims raised in the Petition.[22] *See PGS Geophysical AS v. Iancu*, 891 F.3d 1354, 1360 (Fed. Cir. 2018) (indicating that a decision whether to institute an *inter partes* review "require[s] a simple yes-or-no institution choice respecting a petition, embracing all challenges included in the petition").

Our decision at this stage derives from our preliminary review of the challenged claims, the asserted prior art, and the opinions set forth in the as-yet-unrebutted Balthasar Declaration. We emphasize that at this stage of the proceeding, we have not made a final determination as to the construction of any claim term or the patentability of the instituted claims. Our final decision will be based on the full record developed during trial.

[22] In view of the complexity of the art and arguments presented, the parties are, nevertheless, invited to negotiate an agreement to focus on some subset of the asserted claims and Grounds.

III. ORDER

Accordingly, it is hereby:

ORDERED that, pursuant to 35 U.S.C. § 314, an *inter partes* review of claims 1–3 of U.S. Patent No. 9,718,880 B2, in accordance with each ground on which the challenge to each claim is based in the Petition, is hereby *instituted*; and

FURTHER ORDERED that, pursuant to 35 U.S.C. § 314(c) and 37 C.F.R. § 42.4(b), *inter partes* review of the '880 patent will commence on the entry date of this Order, and notice is hereby given of the institution of a trial.

For PETITIONER:

Deborah A. Sterling
David H. Holman
Scott A. Schaller
David W. Roadcap
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
dsterling-PTAB@ sternekessler.com
dholman-PTAB@sternekessler.com
sschalle-PTAB@ sternekessler.com
droadcap-PTAB@sternekessler.com

For PATENT OWNER:

Gerald J. Flattmann, Jr.
Lori A. Gordon
Vanessa Y. Yen
Evan D. Diamond
James T. Evans
KING & SPALDING LLP
gflattmann@kslaw.com
lgordon@kslaw.com
jevans@kslaw.com